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Analytical Method For Detecting Pcb Derivatives At Low Levels In Surface Water Samples By Solid Phase Extraction-Liquid Chromatography/Mass Spectrometry

Shannon Recca Alford

ANALYTICAL METHOD FOR DETECTING PCB DERIVATIVES AT LOW LEVELS IN SURFACE WATER SAMPLES BY SOLID PHASE EXTRACTION-LIQUID CHROMATOGRAPHY/ MASS SPECTROMETRY

By

Shannon Recca Alford

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Polychlorinated biphenyls (PCBs) and their metabolic derivatives are ubiquitous environmental contaminants. These compounds are of concern because of their persistence and bioaccumulation in nature. PCBs and the hydroxylated metabolites have shown endocrine-disrupting activity. A method of detection in surface water samples is important to identify and quantify the environmental contamination. In this research we have attempted to develop a method of detection. Six representative polychloromethoxybiphenyls (PCMBs) were prepared. The corresponding polychlorobiphenylols, hydroxylated PCB metabolites (OH-PCBs), were prepared from the PCMBs. A method coupling solid phase extraction with liquid chromatography, on-line electrospray ionization, and mass spectrometry (SPE-LC/ESI/MS) was developed for detection of the OH-PCBs in distilled and surface water samples.

DEDICATION

I dedicate this work to my parents, Jerry and Wanda Cooke, who have never failed to encourage me in all my endeavors and have exemplified the importance of a life of learning and to my husband, Jarrod Alford, who has challenged and supported me and without whom I would have never worked towards nor completed this degree.

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LIST OF ABBREVIATIONS

Acetonitrile: ACN, CH_3CN

Atomic mass units: amu

Boron tribromide: BBr_3

Cesium fluoride: CsF

Compound 1: 2',3',3,5-tetrachloro-4-methoxybiphenyl

Compound 2: 3',3,4',5-tetrachloro-4-methoxybiphenyl

Compound 3: 3',3,5',5-tetrachloro-4-methoxybiphenyl

Compound 4: 2',2,3',3,5- pentachloro-4-methoxybiphenyl

Compound 5: 2,3',3,4',5-pentachloro-4-methoxybiphenyl

Compound 6: 2',3',3,5',5- pentachloro-4-methoxybiphenyl

Compound 1H: 2',3',3,5-tetrachloro-4-biphenylol

Compound 2H: 3',3,4',5-tetrachloro-4-biphenylol

Compound 3H: 3',3,5',5-tetrachloro-4-biphenylol

Compound 4H: 2',2,3',3,5- pentachloro-4-biphenylol

Compound 5H: 2,3',3,4',5-pentachloro-4-biphenylol

Compound 6H: 2',3',3,5',5- pentachloro-4-biphenylol

Deuterated chloroform: $CDCl_3$

Dimethyl formamide: DMF

Dimethyl sulfate: DMS

Electrospray ionization: ESI

Ethanol: EtOH

Gas chromatography: GC

Hydrochloric acid: HCl

Infrared spectroscopy: IR

Liquid chromatography: LC

Mass spectrometry: MS

Methylene chloride: CH_2Cl_2

Nuclear magnetic resonance spectroscopy: NMR

Palladium: Pd

Polychlorinated biphenylols: OH-PCBs

Polychlorinated biphenyls: PCBs

Polychlorinated methoxybiphenyls: PCMBs

Potassium iodide: KI

Signal to noise: S/N

Sodium bisulfite: $NaHSO_3$

Sodium carbonate: Na_2CO_3

Sodium hydroxide: NaOH

Sodium sulfate: Na_2SO_4

Solid phase extraction: SPE

Ultraviolet: UV

Water: H_2O

CHAPTER I

INTRODUCTION

General

Polychlorinated biphenyl (PCBs) compounds are well known today as ubiquitous environmental contaminants (1-9). They were commercially produced as dielectrics, cooling fluids, lubricants, and flame retardants, and have been used in hydraulic fluids, adhesives, and plasticizers (5, 10). Production of PCBs was banned in 1976 in the United States and has been banned in other industrialized countries (5, 9). However, the concern surrounding these aromatic compounds arises from their persistence and bioaccumulation in nature (3, 5, 6, 7, 8). Hydroxylated (OH-PCBs) and methoxylated (PCMBs) PCBs are also of concern to the environment.

The OH-PCBs are formed *in vivo* as metabolites of the corresponding PCBs. The mechanism of biotransformation may occur by direct insertion of the hydroxyl group or by epoxide formation (9). The metabolites are formed by enzyme systems known as the cytochrome P450 monooxygenases (8). PCBs are also converted to sulfates or glucuronic acid conjugates in mammals(2). Although the PCBs themselves are not appreciably excreted, the greater polarity of the metabolites facilitates excretion through urine and bile (8, 9). The lipophilic nature of PCBs causes them to bioaccumulate in adipose tissue (9). The OH-PCBs have been found in whole blood, plasma, breast milk,

adipose tissue, and brain tissue(1, 3, 8, 9, 11). PCBs and their metabolites have been noted in various organisms such as bacteria, fungi, rabbits, guinea pigs, rats, mice, monkeys, humans, seals, whales, fish, and eagles (1, 5, 8, 12-15).

Researchers have targeted mammalian, avian, and marine species exposed to these types of contaminants to identify the compounds present and determine the mechanism and effects of the toxicity. This research has indicated endocrine-disrupting activity for PCBs and its derivatives. The effects on the estrogen and thyroid hormone systems are most notable. Some hydroxylated metabolites have a similar structure to thyroxine if the hydroxyl group of the PCB metabolite is in the *para* position to the coupling bond (8, 16). Refer to Figure 1. Thyroxine is an uncommon amino acid and hormone synthesized in the thyroid. It is found in the protein thyroglobulin, which is involved in the endocrine system (16). The structural similarity may allow the metabolite to bind to the thyroxine receptor, and this is believed to be the most likely mechanism of PCB endocrine-disrupting activity (8). It is believed that exposure most often occurs through the animal's diet because PCBs are readily absorbed from the intestine (12). Bottom-dwelling aquatic organisms are exposed through diet and gills (9). Human populations with high fish consumption, such as the Inuit, have high concentrations of PCBs and the corresponding metabolites in whole blood samples (8).

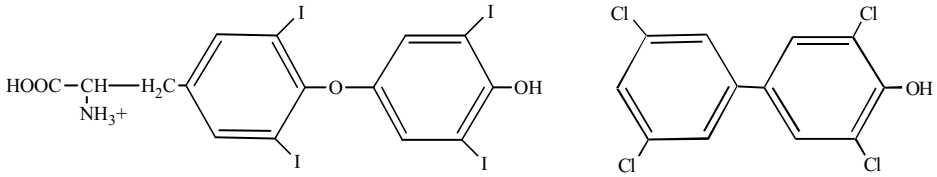


Figure 1: Structural Similarity Between Thyroxine and OH-PCB

Three of the six metabolites used in this research, 3',3,4',5-tetrachloro-4-biphenylol (2, 8, 9, 14, 15, 17), 3',3,5',5-tetrachloro-4-biphenylol (17), and 2,3',3,4',5-pentachloro-4-biphenylol (2, 8, 11, 17, 18), have been previously synthesized and analyzed for toxicity.

Synthesis of PCB Metabolites

The ability to prepare PCBs and the corresponding metabolites is important to further research about how organisms metabolize the contaminants, what effects are caused by the metabolites, and to develop detection methods. This study aimed to prepare six OH-PCBs from the corresponding PCMBs for analytical studies.

The synthesis of PCMBs can be performed using various methods including the Suzuki, Ullman, and Cadogan methods (5, 6). The Suzuki method, used in this research, involves coupling a brominated or iodinated chloroanisole with a chloroaryl boronic acid (or ester) via a palladium catalyst. This method is advantageous because it does not yield much of the by-products arising from self-coupling of the starting materials. The coupling between the aryl rings occurs specifically where the bromine is attached to the anisole and where the boron group is attached to the chlorinated benzene. Suzuki

coupling is less effective when chlorines are present in the *ortho* position to the coupling bond. As the number of chlorines *ortho* to the coupling bond increases, the product yield significantly decreases or the reaction does not occur at all. Suzuki coupling is more time-consuming than the other methods.

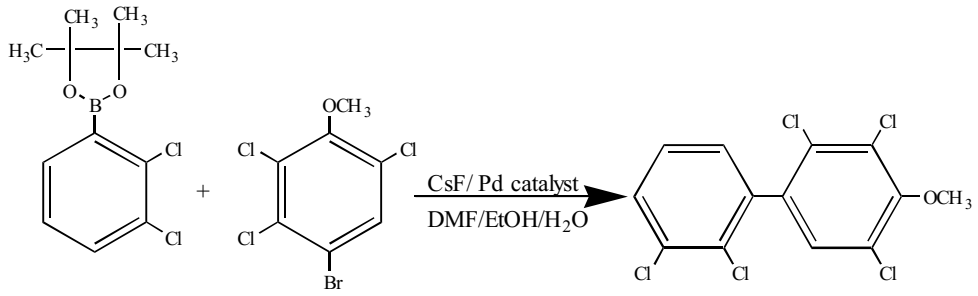


Figure 2: Suzuki Coupling- Preparation of 2',2,3',3,5-pentachloro-4-methoxybiphenyl

The modified Ullmann method, also used by this researcher but not discussed here, is performed by coupling a brominated or iodinated chloroanisole with a brominated or iodinated chlorobenzene using a copper catalyst. The Ullmann method yields all possible coupled products by coupling the rings via the iodine positions, as indicated in Figure 3 below. This reduces the yield of the desired product. However, this reaction requires fewer steps and much less time than the Suzuki method. The Ullmann method also requires that the starting materials have the same active halogen group, either iodine or bromine. The coupling will not occur between brominated and iodinated materials. (3,19,20)

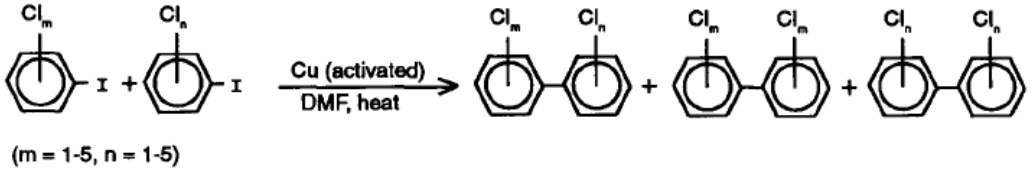


Figure 3: Ullman Coupling

The Cadogan method uses a polychloroaniline and a polychlorobenzene to prepare a PCB via diazotization of the aniline and thermal decomposition of the diazonium salt. This method generates an aryl radical from the diazonium salt of the aniline, which then may substitute at each available hydrogen substituted position on the chlorinated benzene (21). This method can be modified to couple a polychloroaniline with a polychloroanisole to prepare a PCMB, using the same procedure.

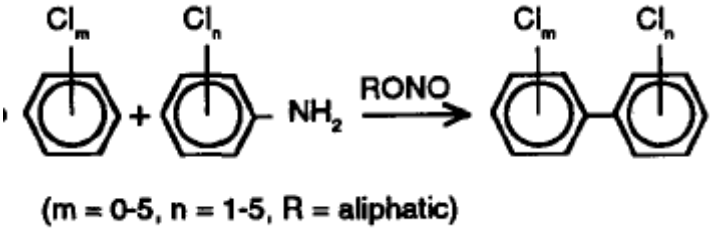


Figure 4: Cadogan Coupling

Tables 1 and 2 contain the assigned numbers, chemical names, and structures of the PCMBs and OH-PCBs, respectively, that were prepared using the Suzuki method.

Table 1: Six PCMBs Prepared by the Suzuki Method

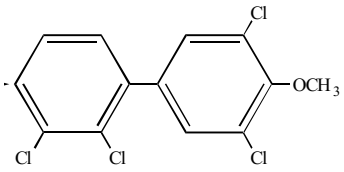
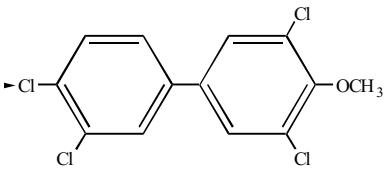
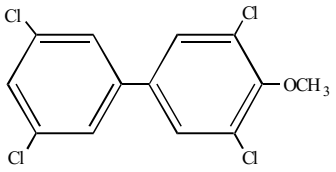
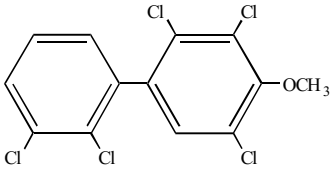
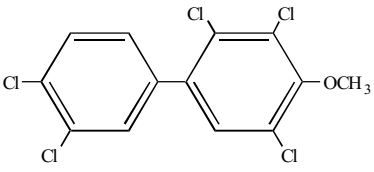
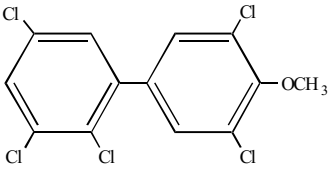
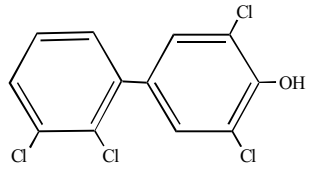
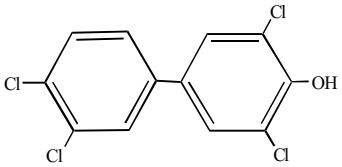
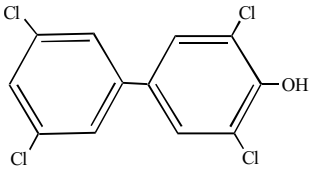
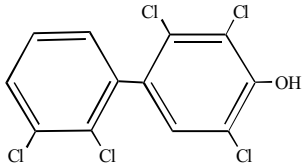
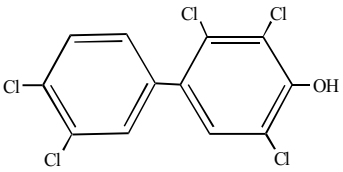
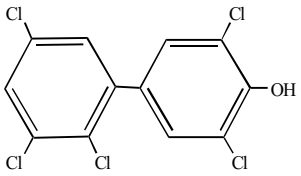
PCMB Number	PCMB Name	PCMB Structure
1	2',3',3,5-tetrachloro-4-methoxybiphenyl	
2	3',3,4',5-tetrachloro-4-methoxybiphenyl	
3	3',3,5',5-tetrachloro-4-methoxybiphenyl	
4	2',2,3',3,5- pentachloro-4-methoxybiphenyl	
5	2,3',3,4',5-pentachloro-4-methoxybiphenyl	
6	2',3',3,5',5- pentachloro-4-methoxybiphenyl	

Table 2: Six OH-PCBs Prepared from PCMBs

OH-PCB Number	OH-PCB Name	OH-PCB Structure
1H	2',3',3,5-tetrachloro-4-biphenylol	
2H	3',3,4',5-tetrachloro-4-biphenylol	
3H	3',3,5',5-tetrachloro-4-biphenylol	
4H	2',2,3',3,5- pentachloro-4-biphenylol	
5H	2,3',3,4',5-pentachloro-4-biphenylol	
6H	2',3',3,5',5- pentachloro-4-biphenylol	

Analytical Methods

Well-defined analytical procedures are important to determine where in the environment the metabolites occur, to identify which metabolites are present, and to estimate their persistence in the environment. The structures of the synthetic PCB metabolites were confirmed by IR, NMR, GC/MS, and from the precedents for how the synthetic reactions occur. The aim of this study was to develop a method for quantitative detection of OH-PCBs in water samples. The OH-PCB compounds were spiked into distilled water and surface water samples and were detected by an SPE-LC/ESI/MS method.

CHAPTER II

EXPERIMENTAL-PREPARATION

Instrumentation

Infrared Spectroscopy (IR)

The IR spectra of all the PCMBs were obtained using a MIDAC M-Series FTIR spectrophotometer in conjunction with PIKE Technologies MIRacle Single Reflection HATR (attenuated total reflectance) accessory for solid samples.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Proton and carbon NMR data were obtained for each of the PCMB compounds in $CDCl_3$ using a Varian Mercury VX 400MHz nuclear magnetic resonance spectrometer.

Gas Chromatography/ Mass Spectrometry (GC/MS)

The identity of all synthesized compounds was confirmed using GC/MS with helium as the carrier gas. A Varian Star 3400 CX gas chromatograph in tandem with a Varian Saturn 3 ion trap mass spectrometer was the instrument most often used for identification. A Varian Star 3600 CX gas chromatograph connected to a Varian Chrompack Saturn 2000 ion trap mass spectrometer was also used. A Varian Star 3400 gas chromatograph was used alone to identify substances when a retention time was

already known. The same GC conditions were used for all instruments. The injector was set at 220°C and the detector was set at 250°C. All samples were dissolved in CH_2Cl_2 for injection.

Procedures

Preparation of chlorinated benzene boronic esters

A general method was used for the preparation of the three boronic pinacol esters used in the later coupling syntheses. The 2,3-dichlorobenzene boronic ester, 3,5-dichlorobenzene boronic ester, and 2,3,5-trichlorobenzene boronic ester (for PCMB compounds 1 and 4, 3, and 6 respectively) were prepared from the commercially available corresponding boronic acids (Aldrich). The boronic acid (1mmol) was dissolved in 30mL of acetone. Pinacol (1.5mmol, 0.177g) was added and the solution was stirred for approximately 20 minutes. The acetone was removed by a rotary-evaporator. The residue was dissolved in ether, washed with water, and dried with Na_2SO_4. The ether was then removed with a rotary-evaporator. The remaining solid was used without further purification so the percent yield was not determined.

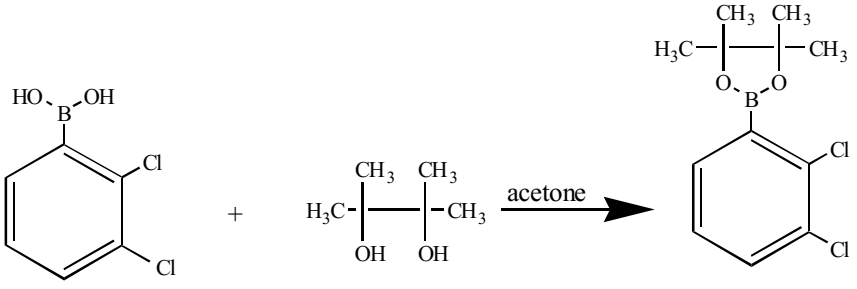


Figure 5: Preparation of 2,3-dichlorobenzene boronic ester

Preparation of 3,4-dichlorobenzene boronic acid

This benzene boronic acid (for PCMB compounds 2 and 5) was available commercially (Aldrich).

Preparation of 4-bromo-2,6-dichloroanisole

The 4-bromo-2,6-dichloroanisole (for PCMB compounds 1,2,3, and 6) was prepared from the commercially available 2,6-dichloroanisole (Aldrich) by modification of the method described by Lehmler and Robertson (5). Bromine (15mL, 2mmol/mL) in glacial acetic acid was added to a solution of 2,6-dichloroanisole (10mmol, 1.76g) in 5mL of glacial acetic acid. The solution was allowed to sit overnight. The reaction progress was monitored by GC/MS. At this time, a solution of 1g of $NaHSO_3$ in 20mL of water was added to the reaction solution to reduce the remaining bromine. A color change from orange to white occurred upon addition of the $NaHSO_3$. The solution was extracted five times with 15mL of methylene chloride. The methylene chloride extracts were washed three times with 20mL water and dried with Na_2SO_4. Residual 2,6-dichloroanisole was still present in the final solution. The 2,6-dichloroanisole was allowed to evaporate. The resulting solid, 0.509g (20% yield), was 100% pure as estimated by GC/MS data (see Figures A.1 and A.3).

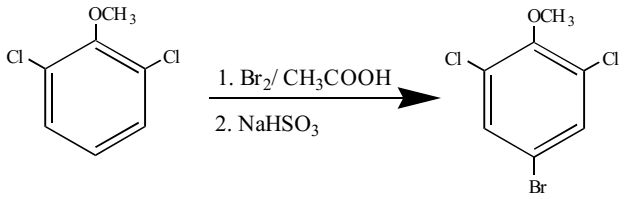


Figure 6: Preparation of 4-bromo-2,6-dichloroanisole

Preparation of 4-bromo-2,3,6-trichlorophenol

The 4-bromo-2,3,6-trichlorophenol (for PCMB compounds 4 and 5) was prepared from commercially available 2,3,6-trichlorophenol (Aldrich) by modification of the method described by Lehmler and Robertson (5). Bromine (10mL, 2mmol/mL) in glacial acetic acid was added to a solution of 2,3,6-trichlorophenol (10mmol, 1.96g) in 5mL of glacial acetic acid. The solution was stirred overnight. The reaction was monitored by GC/MS until no starting material was observed, see Figures A.1 and A.4. A solution of 1g of $NaHSO_3$ in 20mL of water was added to the reaction solution to reduce the bromine. A color change from orange to white occurred upon addition of the $NaHSO_3$. The solution was extracted three times with 15mL of methylene chloride. The solvent was removed by a rotary-evaporator. The yield was not determined because the product was used directly in the following step.

Preparation of 4-bromo-2,3,6-trichloroanisole

The brominated phenol was next methylated. The solid 4-bromo-2,3,6-trichlorophenol was dissolved in 13mL of 2M NaOH. Dimethyl sulfate, DMS, (2.5mL) was added and the solution was stirred for 3 minutes. The solution became a solid and

was allowed to sit overnight. The reaction progress was analyzed by GC/MS the next day. No starting material was observed, see Figures A.1 and A.5. The solution was extracted three times with 20mL of methylene chloride. The extracts were washed twice with 20mL of 0.5M NaOH. The extracts were then dried with anhydrous Na_2SO_4. The solvent was removed by a rotary-evaporator. This reaction yielded 2.420g (84%).

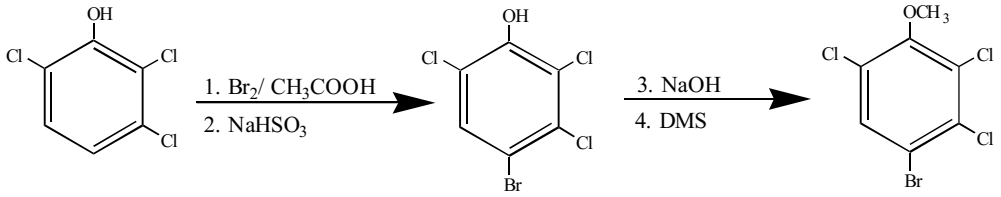


Figure 7: Preparation of 4-bromo-2,3,6-trichloroanisole

Preparation of 2',3',3,5-tetrachloro-4-methoxybiphenyl (Compound 1)

All the PCMBs (Compounds 1-6) were synthesized using a modified Suzuki-type coupling. All synthesis procedures were performed under an argon atmosphere.

Compound 1 (2',3',3,5-tetrachloro-4-methoxybiphenyl) was prepared from 4-bromo-2,6-dichloroanisole (1.15mmol, 0.295g) and 2,3-dichlorobenzene boronic ester (1mmol, 0.273g) with a base, cesium fluoride (3mmol, 0.456g), and 50mg of the catalyst tetrakis(triphenylphosphine)palladium[0] in 10mL dimethyl formamide, 2mL ethanol, and 2mL water. The reaction mixture was stirred and refluxed at 80-90°C for 3hr. The reaction progress was followed by analyzing an aliquot of the mixture using GC/MS to determine the presence of starting materials and reaction products. Once the reaction was determined to be complete, hydrogen peroxide (1mL) was added to convert any

remaining benzene boronic acid to a phenol so it would wash out with the base. Water (10mL) was then added to the mixture. The reaction products were extracted thrice with methylene chloride (10mL), washed with a 2M sodium hydroxide solution (10mL), washed again thrice with water (20mL), and dried with sodium sulfate. Solvent was removed using a rotary-evaporator. The remaining residue was dissolved in boiling hexane, and then filtered to remove undissolved particles. The solvent was removed from the filtrate using a rotary-evaporator. This residue was then dissolved in hexane and purified on a column containing 75mL of silica gel. The column was eluted with hexane. GC/MS was used to monitor the purification procedure on the column. The desired product was collected, and the solvent was evaporated from the product. White needles (132mg) were isolated giving a 39% yield. IR spectroscopy, and carbon and proton NMR spectroscopy were used in conjunction with GC/MS data to confirm the synthesis of the PCMB (compound 1). These data are included and discussed in Chapter IV.

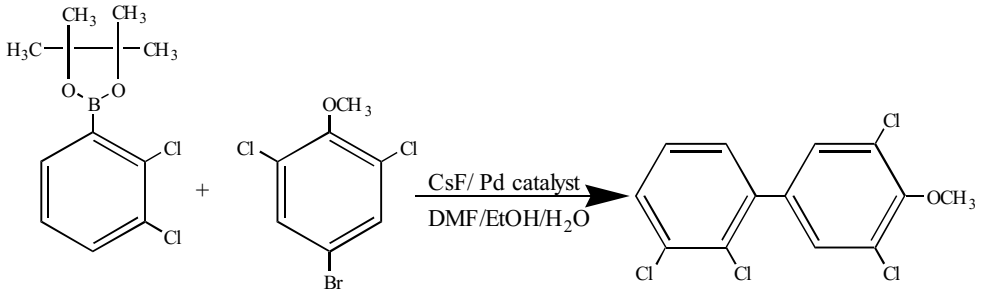


Figure 8: Preparation of 2',3',3,5-tetrachloro-4-methoxybiphenyl (Compound 1)

Preparation of 3',3,4',5-tetrachloro-4-methoxybiphenyl (Compound 2)

Compound 2 (3',3,4',5-tetrachloro-4-methoxybiphenyl) was prepared from 4-bromo-2,6-dichloroanisole (0.200g) and 3,4-dichlorobenzene boronic acid (0.150g) (1:1mmol). The same modified Suzuki-type coupling method and isolation techniques were used as described for the synthesis of compound 1 except for the following differences. Sodium carbonate (2:1mmol ratio to starting material, 0.160g)) was the base used. The reaction solvents were 46mL toluene, 22mL ethanol, and 0.75mL water. The solution was refluxed at 80-90°C for 22hrs. Pale yellow flakes (247mg) were isolated giving a 98% yield. IR spectroscopy, and carbon and proton NMR spectroscopy were used in conjunction with GC/MS data to confirm the synthesis of the PCMB (compound 2). These data are included and discussed in Chapter IV.

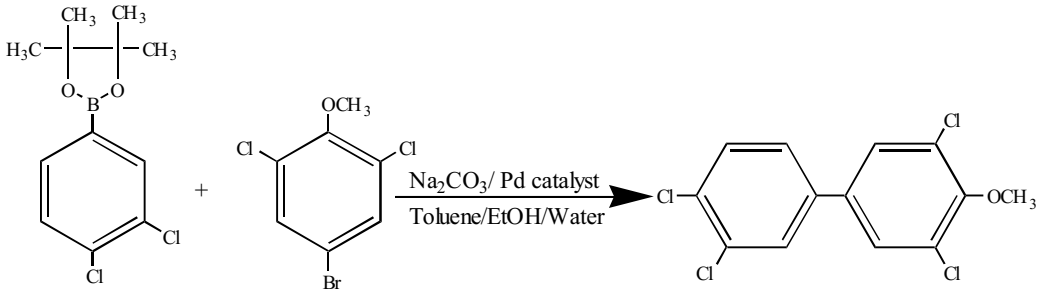


Figure 9: Preparation of 3',3,4',5-tetrachloro-4-methoxybiphenyl (Compound 2)

Preparation of 3',3,5',5-tetrachloro-4-methoxybiphenyl (Compound 3)

Compound 3 (3',3,5',5-tetrachloro-4-methoxybiphenyl) was prepared from 4-bromo-2,6-dichloroanisole (0.295g)and 3,5-dichlorobenzene boronic ester (0.273g) (1.15:1mmol). The same modified Suzuki-type coupling method and isolation techniques

were used as described for the synthesis of compound 1 except for the following differences. Cesium fluoride (3mmol, 0.456g) was the base used. The reaction solvents were 10mL dimethyl formamide, 2mL ethanol, and 2mL water. The solution was refluxed at 80-90°C for 3hrs. White flakes (234mg) were isolated giving a 72% yield. IR spectroscopy, and carbon and proton NMR spectroscopy were used in conjunction with GC/MS data to confirm the synthesis of the PCMB (compound 3). These data are included and discussed in Chapter IV.

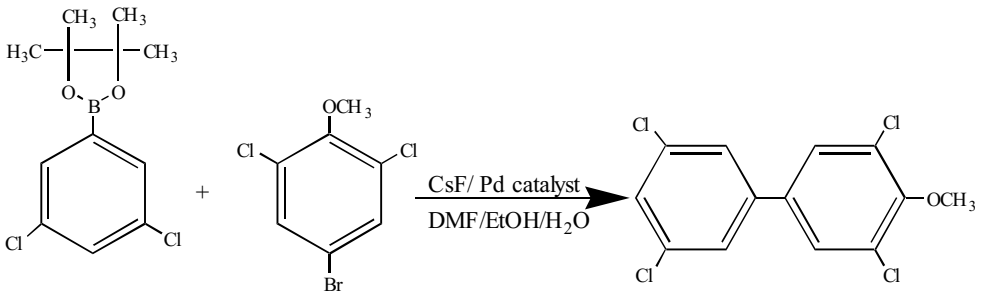


Figure 10: Preparation of 3',3,5',5-tetrachloro-4-methoxybiphenyl (Compound 3)

Preparation of 2',2,3',3,5- pentachloro-4-methoxybiphenyl (Compound 4)

Compound 4 (2',2,3',3,5- pentachloro-4-methoxybiphenyl) was prepared from 4-bromo-2,3,6-trichloroanisole (0.295g) and 2,3-dichlorobenzene boronic ester (0.273g) (1.15:1mmol). The same modified Suzuki-type coupling method and isolation techniques were used as described for the synthesis of compound 1 except for the following differences. Cesium fluoride (3mmol, 0.456g) was the base used. The reaction solvents were 10mL dimethylformamide, 2mL ethanol, and 2mL water. The solution was refluxed at 80-90°C for 4hrs. A white powder (172mg) was isolated giving a 32% yield.

IR spectroscopy, and carbon and proton NMR spectroscopy were used in conjunction with GC/MS data to confirm the synthesis of the PCMB (compound 4). These data are included and discussed in Chapter IV.

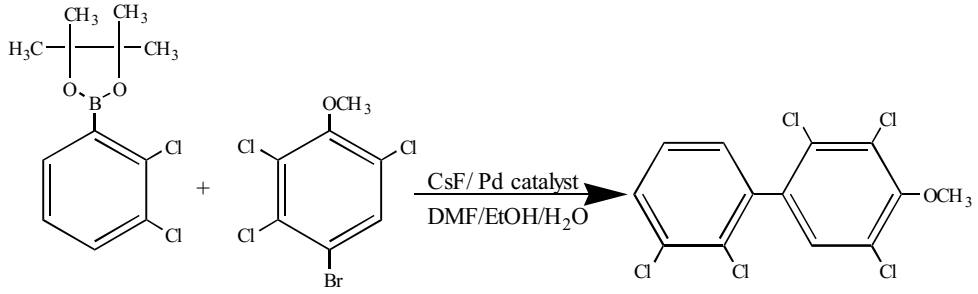


Figure 11: Preparation of 2',2,3',3,5- pentachloro-4-methoxybiphenyl (Compound 4)

Preparation of 2,3',3,4',5-pentachloro-4-methoxybiphenyl (Compound 5)

Compound 5 (2,3',3,4',5-pentachloro-4-methoxybiphenyl) was prepared from 4-bromo-2,3,6-trichloroanisole (0.300g) and 3,4-dichlorobenzene boronic acid (0.197g) (1:1mmol). The same modified Suzuki-type coupling method and isolation techniques were used as described for the synthesis of compound 1 except for the following differences. Sodium carbonate (2mmol, 0.212g)) was the base used. The reaction solvents were 17.5mL toluene, 10mL ethanol, and 1mL water. The solution was refluxed at 80-90°C for 16hrs. Light brown flakes (291mg) were isolated giving an 82% yield. IR spectroscopy, and carbon and proton NMR spectroscopy were used in conjunction with GC/MS data to confirm the synthesis of the PCMB (compound 5). These data are included and discussed in Chapter IV.

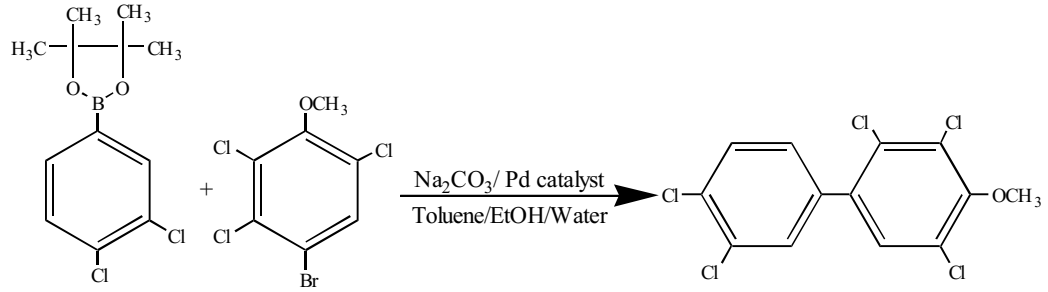


Figure 12: Preparation of 2,3',3,4',5-pentachloro-4-methoxybiphenyl (Compound 5)

Preparation of 2',3',3,5',5- pentachloro-4-methoxybiphenyl (Compound 6)

Compound 6 (2',3',3,5',5- pentachloro-4-methoxybiphenyl) was prepared from 4-bromo-2,6-dichloroanisole (0.294g) and 2,3,5-trichlorobenzene boronic ester (0.307g) (1.15:1mmol). The same modified Suzuki-type coupling method and isolation techniques were used as described for the synthesis of compound 1 except for the following differences. Cesium fluoride (3mmol, 0.456g) was the base used. The reaction solvents were 10mL dimethyl formamide, 2mL ethanol, and 2mL water. The solution was refluxed at 80-90°C for 4hrs. A white powder (40mg) was isolated giving an 11% yield. IR spectroscopy, and carbon and proton NMR spectroscopy were used in conjunction with GC/MS data to confirm the synthesis of the PCMB (compound 6). These data are included and discussed in Chapter IV.

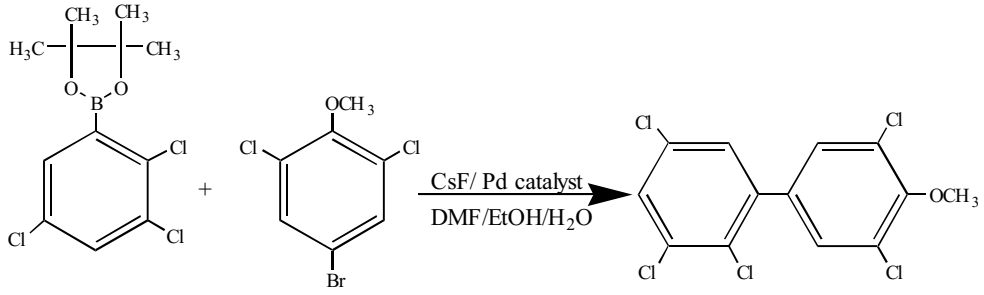


Figure 13: Preparation of 2',3',3,5',5- pentachloro-4-methoxybiphenyl (Compound 6)

Preparation of 2',3',3,5-tetrachloro-4-biphenylol (Compound 1H)

Compound 1H (2',3',3,5-tetrachloro-4-biphenylol) was prepared from 20mg of compound 1. All synthesis procedures were performed under an argon atmosphere. Compound 1 was dissolved in 5mL of methylene chloride, and 2mL of 1M boron tribromide in dichloromethane was added to the solution to demethylate compound 1. The reaction solution was allowed to sit overnight. Approximately 20mL of water was added to the solution. The reaction products were extracted with ethyl ether, washed with water, and purified on a silica solid phase extraction cartridge (Supelco, Supelclean LC-SI 6mL tube). The products were eluted with ethyl ether. The fractions were monitored by GC/MS. GC/MS data were used to confirm the synthesis of the OH-PCB (compound 1H). These data are included and discussed in Chapter IV. The solid product was obtained by removing the solvent with a rotary-evaporator. The compound was not further purified, but the purity was estimated by GC/MS. Approximation by GC/MS indicated 93% purity. A solid (14mg) was isolated giving a 68% yield.

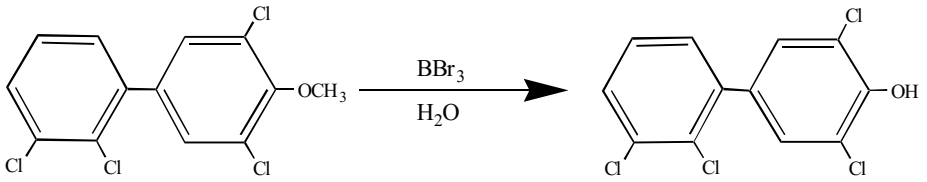


Figure 14: Preparation of 2',3',3,5-tetrachloro-4-biphenylol (Compound 1H)

Preparation of 3',3,4',5-tetrachloro-4-biphenylol (Compound 2H)

Compound 2H, 3',3,4',5-tetrachloro-4-biphenylol, was prepared using the same demethylation and isolation procedures as described for compound 1H except for the following differences. Compound 2H was prepared from 40mg of compound 2. Approximation by GC/MS indicated 100% purity. Compound 2H (17mg) was isolated giving a 43% yield. GC/MS data were used to confirm the synthesis of the OH-PCB (compound 2H). These data are included and discussed in Chapter IV.

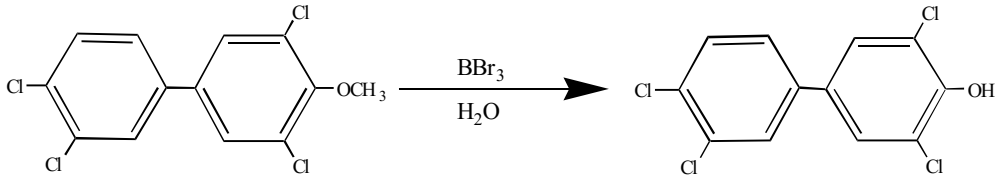


Figure 15: Preparation of 3',3,4',5-tetrachloro-4-biphenylol (Compound 2H)

Preparation of 3',3,5',5-tetrachloro-4-biphenylol (Compound 3H)

Compound 3H, 3',3,5',5-tetrachloro-4-biphenylol, was prepared using the same demethylation and isolation procedures as described for compound 1H except for the following differences. Compound 3H was prepared from 40mg of compound 3. GC/MS

indicated 92% purity. A solid (41mg) was isolated giving a 99% yield. GC/MS data were used to confirm the synthesis of the OH-PCB (compound 3H). These data are included and discussed in Chapter IV.

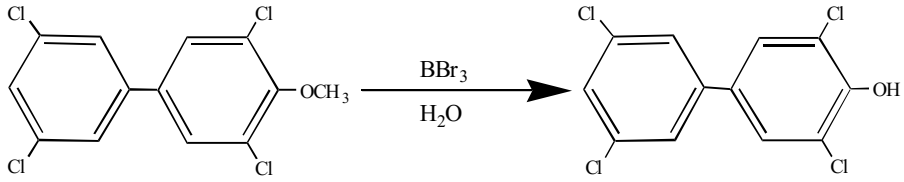


Figure 16: Preparation of 3',3,5',5-tetrachloro-4-biphenylol (Compound 3H)

Preparation of 2',2,3',3,5- pentachloro-4-biphenylol (Compound 4H)

Compound 4H, 2',2,3',3,5- pentachloro-4-biphenylol, was prepared using the same demethylation and isolation procedures as described for compound 1H except for the following differences. Compound 4H was prepared from 40mg of compound 4. Approximation by GC/MS indicated 89% purity with some compound 4 still remaining. A solid (36mg) was isolated giving an 83% yield. GC/MS data were used to confirm the synthesis of the OH-PCB (compound 4H). These data are included and discussed in Chapter IV.

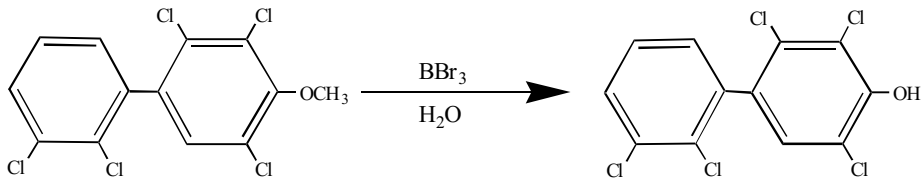


Figure 17: Preparation of 2',2,3',3,5- pentachloro-4-biphenylol (Compound 4H)

Preparation of 2,3',3,4',5-pentachloro-4-biphenylol (Compound 5H)

Compound 5H, 2,3',3,4',5-pentachloro-4-biphenylol, was prepared using the same demethylation and isolation procedures as described for compound 1H except for the following differences. Compound 5H was prepared from 40mg of compound 5. Approximation by GC/MS indicated 100% purity. Compound 5H (35mg) was isolated giving a 91% yield. GC/MS data were used to confirm the synthesis of the OH-PCB (compound 5H). These data are included and discussed in Chapter IV.

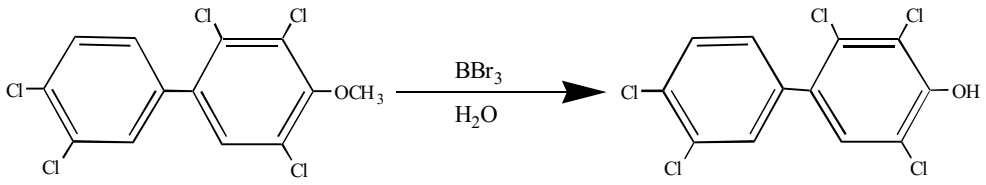


Figure 18: Preparation of 2,3',3,4',5-pentachloro-4-biphenylol (Compound 5H)

Preparation of 2',3',3,5',5- pentachloro-4-biphenylol (Compound 6H)

Compound 6H, 2',3',3,5',5- pentachloro-4-biphenylol, was prepared using the same demethylation and isolation procedures as described for compound 1H except for the following differences. Compound 6H was prepared from 16mg of compound 6. Approximation by GC/MS indicated 100% purity. Compound 6H (15mg) was isolated giving a 100% yield. GC/MS data were used to confirm the synthesis of the OH-PCB (compound 6H). These data are included and discussed in Chapter IV.

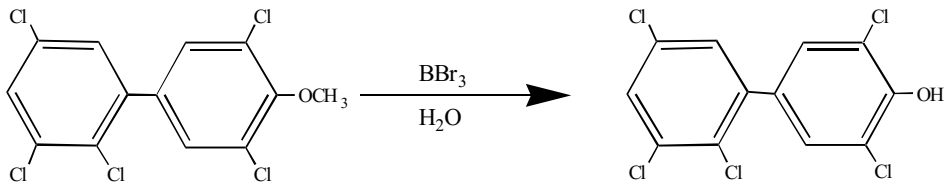


Figure 19: Preparation of 2',3',3,5',5- pentachloro-4-biphenylol (Compound 6H)

CHAPTER III

EXPERIMENTAL- ANALYTICAL METHODS

Instrumentation

Liquid Chromatography/ Mass Spectrometry (LC/MS)

A Varian ProStar binary LC system (Varian Inc., Walnut Creek, CA) was attached to a Bruker Esquire mass spectrometer (Bruker Daltonics Inc., Billerica, MA). Sample volumes injected were 50□L, 100□L, or 200□L taken by the autosampler. The LC column was an Alltech C-18 with an inner diameter of 4mm, 250mm in length, and 5 micron particle size. The mobile phase was acetonitrile (ACN) and water, and 0.1% formic acid was added to both solvents. Several ratios of ACN:H_2O were used. A flow rate of 0.9mL/min was used for the column separation. UV detection was with a Rainin Dynamax UV-1 absorbance detector at □=254nm in series with the MS. Post column infusion of base solution, 10% ammonium hydroxide (NH_4OH) in 45% water and 45% acetonitrile (CH_3CN), was performed via a secondary isocratic Spectroflow 400 Pump (ABI Analytical Kratos Division), supplying a flow of 0.1mL/min (23).The effluent was then subjected to electrospray ionization (ESI), where the high pH of the solution enhanced the production of anions. The ions were analyzed on an Esquire LC Mass Spectrometer (MS) set in negative mode. The MS scan was set to select for ions m/z 308.5 ±1.5 and m/z 342.5 ±1.5.

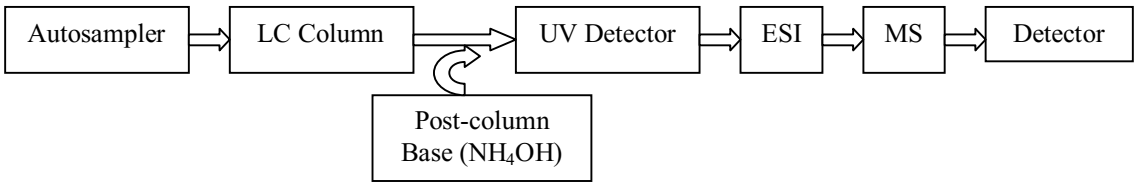


Figure 20: Block Diagram of LC/MS Instrumentation

Solid Phase Extraction (SPE)

All solvents were of OPTIMA^{TM} grade purchased from Fisher Scientific. The solid phase extractions were performed using Supelco-Supelclean LC-18 3mL tubes (Lot No. SP2517H).

Procedures

Stock Solutions/ Dilutions

Table 3 describes stock solutions made of the OH-PCBs (refer to Table 2 for structures) and further dilutions made from these stocks. Experiments were performed using these stock solutions and further dilutions. Relative retention times of each of the compounds were determined using dilution 1 of each compound. Solid phase extraction (SPE) experiments were performed as part of the analysis of surface water samples. The SPE experiments were performed with the stock solution of compound 4H and dilution 1 of all six OH-PCBs in acetonitrile. Standard curves were plotted in area counts versus nanograms of compound using dilutions 1, 2, 2-3, 3, and 4 in acetonitrile and in water. Dilution 1-2 in Table 4 for acetonitrile and dilution 1-2 in Table 5 for water were not included in the standard curves. These dilutions (1-2) were prepared and analyzed on a

different day than the other dilutions for the standard curve data. These two data points fell outside the standard curves obtained from the other dilutions. These data indicate that it is necessary to perform a standard curve analysis each day that the method is used for accurate determination of these analytes. The precision of the LC/MS method was confirmed by reproducibility studies using dilution 2-3 in acetonitrile and dilution 2 in water. See the following Tables 3-5 for the concentrations of each solution.

Table 3: Concentrations for Retention Time
and Solid Phase Extraction Experiments

Compound	Stock solution in ACN	Dilution 1 (10:1) in ACN
1H	50□g/mL	5.0□g/mL
2H	80□g/mL	8.0□g/mL
3H	51□g/mL	5.1□g/mL
4H	55□g/mL	5.5□g/mL
5H	62□g/mL	6.2□g/mL
6H	93□g/mL	9.3□g/mL

Table 4: Concentrations in Acetonitrile for Standard Curves, Reproducibility Studies, and Solid Phase Extraction Experiments

Dilution Label	Dilution	Dilution in Acetonitrile	Concentration in □g/mL					
			1H	2H	3H	4H	5H	6H
1	10:1	100□L of each 6 stocks + 400□L ACN	5	8	5.1	5.5	6.2	9.3
1-2	2:1	300□L of diln 1 + 300□L ACN	□□□□	4	2.55	2.75	□□□□	4.65
2	10:1	100□L of diln 1 +900□L ACN	0.5	0.8	0.51	0.55	0.62	0.93
2-3	4:1	250□L of diln 2 +750□L ACN	0.125	0.2	0.1275	0.1375	0.155	0.2325
3	10:1	100□L of diln 2 +900□L ACN	0.05	0.08	0.051	0.055	0.062	0.093
4	10:1	100□L of diln 3 +900□L ACN	0.005	0.008	0.0051	0.0055	0.0062	0.0093

Table 5: Concentrations in Water for Standard Curves and Reproducibility Studies

Dilution Label	Dilution	Dilution in Water	Concentration in □g/mL					
			1H	2H	3H	4H	5H	6H
1	100:1	10□L of each 6 stocks + 940□L H2O	0.5	0.8	0.51	0.55	0.62	0.93
1-2	2:1	250□L of diln 1 +250□L H2O	□□□□	0.4	0.255	0.275	□□□□	0.465
2	10:1	100□L of diln 1 + 900□L H2O	0.05	0.08	0.051	0.055	0.062	0.093
2-3	4:1	250□L of diln 2 +750□L H2O	0.0125	0.02	0.0128	0.0138	0.0155	0.0233
3	10:1	100□L of diln 2 + 900□L H2O	0.005	0.008	0.0051	0.0055	0.0062	0.0093

Retention Time Experiment

Dilution 1 for each of the OH-PCBs was analyzed separately by LC/MS to determine retention times. All the samples were analyzed with 50□L on the column, with

a 75:25 ratio of ACN:H_2O flowing through the column, with post-column base flowing at 0.1mL/min, and the MS set in negative mode.

Standard Curve Experiment

The six OH-PCBs (refer to Table 2 for structures) were combined to obtain standard curve data at decreasing concentrations, using dilutions in Table 4 and Table 5. Two solvent systems were used for dissolution of the stock standards, 100% ACN and H_2O + ACN. In the H_2O + ACN system, the initial ratio of H_2O:ACN was 94:6. The ratio of H_2O:ACN increased with decreasing compound concentration because the dilutions were made with water. The samples in acetonitrile were all analyzed with 100□L on the column, with a 75:25 ratio of ACN:H_2O flowing through the column, with post-column base flowing at 0.1mL/min, and the MS set in negative mode. The samples in water were analyzed with 200□L injections on the column, with all other conditions the same as above.

Reproducibility Studies

The six OH-PCBs (refer to Table 2 for structures) were analyzed together for the reproducibility studies in both solvent systems, 100% acetonitrile with dilution 2-3 from Table 4 and 10% acetronitrile:90% water with dilution 2 from Table 5. The compounds dissolved in 100% acetonitrile and the compounds dissolved in 10% acetronitrile:90% water were analyzed in exactly the same manner as in the standard curves experiments above.

Solid Phase Extraction (SPE)

Eight experiments were performed to devise a method for detecting OH-PCBs (refer to Table 2 for structures) in surface water samples. Once the samples were eluted from the SPE cartridge in 100% acetonitrile, they were analyzed with 200□L injections on the column, with all other conditions exactly the same as in the standard curve experiments above.

Experiment 1 (Spiked Cartridge): A SPE tube was conditioned with 3mL of acetonitrile, and then with 3mL of water. An aliquot of water, approximately 0.5mL, above the bed of the tube was spiked with 550ng of compound 4H (10□L of the stock solution in acetonitrile). The tube was washed with 10mL of water by applying a vacuum. The compound was eluted from the tube with 5mL of acetonitrile and analyzed as described above.

Experiment 2 (Spiked Cartridge, Large Water Volume): An SPE tube was conditioned with 3mL of acetonitrile, and then with 3mL of water. An aliquot of water, approximately 0.5mL, above the bed of the tube was spiked with 550ng of compound 4H (10□L of the stock solution in acetonitrile). The tube was washed with 125mL of water by applying a vacuum. The compound was eluted from the tube with 5mL of acetonitrile. The eluent solution was diluted with 5mL of water and analyzed as described above.

Experiment 3 (Spiked Cartridge, Concentrated Sample): An SPE tube was conditioned with 3mL of acetonitrile, and then with 3mL of water. An aliquot of water, approximately 0.5mL, above the bed of the tube was spiked with 10□L of the dilution 1

in acetonitrile, containing the 6 OH-PCBs. The amount was approximately 50-100ng of each compound in the spiked sample. The tube was washed with 10mL of water by applying a vacuum. The compound was eluted from the tube with 5mL of acetonitrile. The eluent was concentrated to approximately 200□L and diluted to 500□L with water and analyzed as described above.

Distilled Water Control: A SPE tube was first conditioned with 3mL of acetonitrile, and then with 3mL of water. 25mL of distilled water was passed through the tube. The analytes were eluted from the tube with 5mL of acetonitrile and collected in a glass vial. The eluent was concentrated to approximately 200□L and diluted to 500□L with water and analyzed as described above.

Experiment 4-1 (Spiked Solution, Glass Vial): A 25mL solution was prepared of distilled water and spiked with 10□L of dilution 1 in acetonitrile, containing the 6 OH-PCBs. The amount was approximately 50-100ng of each compound in the spiked sample. An SPE tube was conditioned with 3mL of acetonitrile, and then with 3mL of water. The 25mL of sample solution was passed through the tube. The compound was eluted from the tube with 5mL of acetonitrile and collected in a glass vial. The eluent was concentrated to approximately 200□L and diluted to 500□L with water and analyzed as described above.

Experiment 4-2 (Plastic Vial): Experiment 4-1 was repeated, but the eluent was collected in a plastic vial.

Experiments 5-1 and 5-2 (Surface Water Studies, Filtered): Surface water samples were taken from two local sites: a flowing creek (5-1) and a rain overflow area (5-2). For

both samples, 25mL of the surface water sample was filtered with a 25mm Millipore Millex-LCR Syringe Driven Filter Unit of 45□m pore size. Each sample was spiked with 10□L of dilution 1 in acetonitrile, containing the 6 OH-PCBs. The amounts were approximately 50-100ng of each compound in the spiked sample. An SPE tube was conditioned with 3mL of acetonitrile, and then with 3mL of water. The 25mL of sample solution was passed through the tube. The compound was eluted from the tube with 5mL of acetonitrile and collected in a glass vial. The eluent was concentrated to approximately 200□L and diluted to 500□L with water and analyzed as described above.

Surface Water Controls: For both surface waters, 25mL of the surface water sample was subjected to exactly the same procedure as in the previous experiment except the samples were not spiked and the eluent was concentrated to 100□L and diluted to 500□L with water.

Experiment 5-3 (Particulate Binding, Unfiltered): A surface water sample (25mL) from a flowing creek was spiked with 10□L of dilution 1 in acetonitrile, containing the 6 OH-PCBs. The amounts were approximately 50-100ng of each compound in the spiked sample. An SPE tube was conditioned with 3mL of acetonitrile, and then with 3mL of water. The 25mL of sample solution was passed through the tube. The compound was eluted from the tube with 5mL of acetonitrile and collected in a glass vial. The eluent was concentrated to approximately 200□L and diluted to 500□L with water and analyzed as described above.

CHAPTER IV

RESULTS/DISCUSSION- STRUCTURE ASSIGNMENT

Gas Chromatography/ Mass Spectrometry Data

The observed fragments and the losses leading to the observed ions of the PCMBs from GC/MS are shown in Table 6 (refer to Table 1 for structures). The complete spectrum for each compound is included in Appendix A. Compound 2 has been previously characterized in the literature. Its major fragments and losses leading to the observed ions are reported below (2, 9). Compound 5 has also been characterized and its major fragments assigned(2). Haraguchi has made structure assignments for 2,3',4',5-tetrachloro-4-methoxybiphenyl and 2',2,4',5',5-pentachloro-4-methoxybiphenyl based on the major fragments and losses leading to the ions observed. The observed fragments for the six PCMBs in this study are consistent with those observed by Haraguchi for tetrachloro and pentachloro methoxybiphenyls (4). The reported mass of each of these ions is the lowest mass ion of the chlorine isotope cluster for that fragment or molecular ion.

Table 6: Major Fragments of PCMBs Determined by Mass Spectrometry

Compound #	Retention Time (min)	M^+	M^+-CH_3	M^+-$COCH_3$	M^+-$COCH_3Cl$	M^+-$COCH_3Cl_2$	M^+-$COCH_3Cl_3$
1	14.83	320	305	277	241	207	171
2	13.95	320	305	277	241	207	171
3	15.08	320	305	277	241	207	171
4	13.31	354	339	311	275	241	206
5	16.33	354	339	311	275	241	206
6	15.96	354	339	311	275	241	206

GC/MS data in Table 7 show the observed fragments and molecular ions from the six OH-PCBs (refer to Table 2 for structures) and proposed losses from molecular ions. The complete spectrum for each OH-PCB is included in Appendix A. These ions are consistent with expected results for tetra and pentachloro hydroxybiphenyls.

Table 7: Major Fragments of OH-PCBs Determined by Mass Spectrometry

Compound #	Retention Time (min)	M^+	M^+-HCl	M^+-HCl_2	M^+-COH_2Cl_2	M^+-COH_2Cl_3	M^+-COH_2Cl_4
1H	14.78	306	270	236	207	172	137
2H	15.63	306	270	236	207	172	137
3H	15.06	306	270	236	207	172	137
4H	15.76	340	305	270	241	207	171
5H	16.61	340	305	270	241	207	171
6H	16.00	340	305	270	241	207	171

Structure assignments and therefore the successful preparation of the PCMBs and OH-PCBs were in part established based on the characteristic fragmentation of the molecules, correct relative intensities of the chlorine isotopic clusters, and the presence of the expected molecular parent ion.

Infrared Spectroscopy Data

The following table includes the major peaks in the IR spectra for compounds 1-6 (refer to Table 1 for structures). The complete spectrum for each compound is included in Appendix B. There are several functional groups for which we expect peaks in the IR spectra for each of the compounds. The aromatic-hydrogen vibrations appear between 3100-3000 cm^{-1} (24). Several weak peaks are visible in this area of the spectra for all six of the PCMBs. The carbon-carbon stretching in the aromatic rings should have medium peaks in the range of 1600-1450cm^{-1} (25). A few peaks are visible in this region for each of the compounds. The methoxy group on the aromatic ring should have a peak in the ranges of 1800-1300cm^{-1}, for the aryl-O stretching, and 1070-970cm^{-1}, for the CH_3-O stretch (26). There are peaks in the 1400cm^{-1} range and a strong peak at approximately 990cm^{-1} or 1000cm^{-1} for all the PCMBs that can be attributed to the methoxy group. Peaks for out-of-plane aromatic C-H vibrations occur in the range of 900-700cm^{-1} (26, 27). Each of the PCMBs has some peaks in this range, which can be assigned to these aromatic C-H bonds. Finally, we would expect to see peaks in the range of 800-600cm^{-1} for the C-Cl bonds in the PCMBs (25). There is a strong peak at approximately 800cm^{-1} for all the PCMBs that can be assigned to the C-Cl bonds.

Table 8: IR Data for PCMBs

	PCMB Compound					
	1	**2**	**3**	**4**	**5**	**6**
Major Peaks (cm^{-1}) in the Ar-H region	3069	3070	3077	3014	3004	3055
	3059	3006	3002	2992	2991	3004
	3005	2959	2994	2952	2951	2990
	2992	2942	2952	2922	2923	2948
	2950	2870	2872	2851	2851	2922
	2836	2854	2853			2852
		2834	2835			
Major Peaks (cm^{-1}) in the fingerprint region	1416	1462	1544	1409	1455	1483
	1370	1258	1487	1001	1135	1257
	1260	992	1418	830	1004	990
	990	869	1355	781	801	750
	809	803	984	643	612	658
	772	685	850			
	653		805			
			621			

Nuclear Magnetic Resonance Spectroscopy Data

Table 9 includes the ^{1}H-NMR data from this study and proton shift assignments for the six PCMBs (refer to Table 1 for structures). Although only compound 4 has been characterized in literature (9), several compounds with at least one ring identical to the ones in this study have been characterized by ^{1}H-NMR. Refer to Figure 21 for an example of the proton and carbon numbering system for the PCMBs. The proton resonances were assigned based on the assignments made by Shiraishi, Lehmler, Haraguchi, and van den Hurk, which are summarized in Table 10 (4, 5, 9, 10). The observed shifts, magnitude of the coupling constants, and multiplicities agree very well with those referenced. The methoxy hydrogens have a singlet peak at 3.9ppm for all six PCMBs and this value agrees with literature values in Table 10. Singlet peaks for H-2 and H-6 are also seen for the six PCMBs. H-2 and H-6 are equivalent for compounds 1,2,3, and 6 and are therefore assigned the same singlet peak shift. The hydrogens on the non-methoxylated ring were all assigned based on the magnitude of the coupling constants and shift values for similar compounds, seen in Table 10.

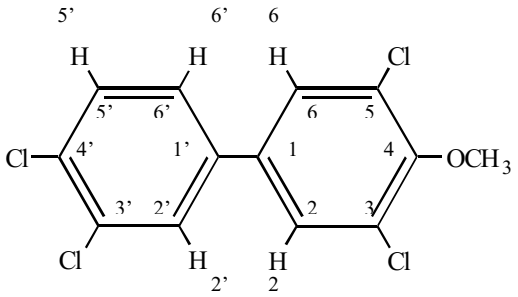


Figure 21: Numbering System for Compound 2

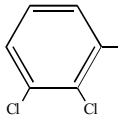
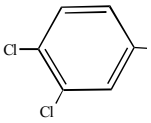
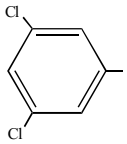
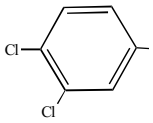
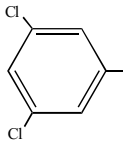
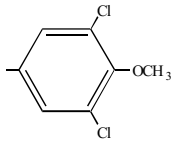
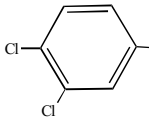
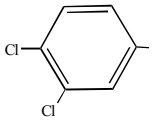
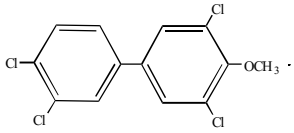
Table 9: ^{1}H- NMR Data for PCMBs

PCMB Compound	OCH_3	H-2	H-6	H-2'	H-3'	H-4'	H-5'	H-6'
1	s 3.9	s 7.35	s 7.35	~	~	d,d 7.49 l,sm	q 7.27 l,l	d,d 7.19 l,sm
2	s 3.9	s 7.46	s 7.46	d 7.51 sm	~	~	d 7.58 l	d,d 7.33 l,sm
3	s 3.9	s 7.46	s 7.46	d 7.36 sm	~	d 7.37 sm	~	d 7.36 sm
4	s 3.9	~	s 7.23	~	~	d,d 7.54 l,sm	t 7.28 l,l	d,d 7.13 l,sm
5	s 3.9	~	s 7.28	d 7.47 sm	~	~	d 7.51 l	d,d 7.22 l,sm
6	s 3.9	s 7.34	s 7.34	~	~	d 7.51 sm	~	d 7.21 sm

s=singlet, d=doublet, t=triplet, q=quartet Shift values given in ppm.

coupling constants l=large (5-10 Hz), sm=small (2-4 Hz)

Table 10: ^{1}H- NMR Shift Assignments from Literature

Reference/ Compound	Structure	OCH3	H-2	H-6	H-2'	H-3'	H-4'	H-5'	H-6'
Shiraishi/ 1&4		~	~	~	~	~	7.508	7.255	7.153
Shiraishi/ 2&5		~	~	~	7.414	~	~	7.564	7.148
Shiraishi/ 3		~	~	~	7.198	~	7.436	~	7.198
Lehmler/ 2&5		s 3.92	~	~	d 7.56	~	~	d 7.44	d,d 7.30
Lehmler/ 3		s 3.79	~	~	d 7.36	~	t 7.31	~	d 7.36
Lehmler/ 1,2,3,6		s 3.97	s 7.36	s 7.36	~	~	~	~	~
Haraguchi/ 2&5		~	~	~	d 7.5	~	~	d 7.5	d,d 7.25
van den Hurk/ 2&5		s 3.92	s 7.52	s 7.52	d 7.65	~	~	d 7.54	d,d 7.39
van den Hurk/ 2		s 3.94	~	~	d 7.5	~	~	d 7.52	d,d 7.27

s= singlet, d= doublet, t= triplet Shift values given in ppm.

Table 11 includes data for ^{13}C- NMR for the six PCMBs in this study (refer to Table 1 for structures). The carbons were not individually assigned shift values, however it was possible to determine the presence of a methoxy carbon at 61ppm for five of the compounds. It is unclear why compound 6 does not have a peak at 61ppm. It is evident that compound 6 has a methoxy group based on the ^{1}H- NMR data. There are also only four other peaks representative of the aromatic carbons for compound 6. This indicates that perhaps the sample was too dilute to accurately determine the structure of compound 6 with ^{13}C- NMR. In fact, all the compounds except compound 5 indicate that the sample may have been too dilute to see all the carbons. The spectrum for compound 5 contains more than twelve peaks in the aromatic range. The extra peaks are most likely present because of impurities in this sample. The values ranging from 125-135ppm for the shifts of all the PCMBs are evidence that the carbons are aromatic, which should have shifts in the range of 110-175ppm (27). The quality of the ^{13}C- NMR spectra was not good enough to strongly support the assigned structures; however, they were, for the most part, not inconsistent with those assignments.

Table 11: ^{13}C- NMR Data for PCMBs

PCMB Compound	1	2	3	4	5	6
OCH_3	61	61	61	61	61	
Aromatic Carbons	127.4	126.0	125.415	127.299	127.5	129.23
	129.1	127.1	127.413	129.076	128.5	129.27
	129.2	128.5	128.039	129.541	128.7	129.60
	129.8	130.0	130.037	130.647	129.5	129.90
	130.4	131.0	135.612	132.058	130.5	
	133.9	132.1	135.841	133.545	131.2	
	136.4	132.5	141.157	135.757	132.1	
	139.9	133.1	152.490	138.968	132.6	
		136.0		153.238	136.2	
		138.2		159.156	137.9	
		152.2			153.5	
					156.8	

Shift values given in ppm.

The combined GC/MS, IR, and ^{1}H- NMR data indicate that the structural assignments for the six PCMBs and the corresponding OH-PCBs were correctly made. The ^{13}C- NMR data were inconclusive but tended to support these assignments also.

CHAPTER V

RESULTS/DISCUSSION- ANALYTICAL METHODS

Liquid Chromatography

The chromatographic response was optimized by altering several conditions of the separation. A solution of 1% formic acid was included in the acetonitrile and water mobile phase solvents. The formic acid should be effective in maintaining the protonated state of the acidic biphenylols on the column. The ACN:H_2O ratio, addition of post-column base, and the amount of sample were varied.

There are two pairs of congeners that were not separated well chromatographically. Compounds 1H and 4H have very similar retention times as do compounds 5H and 6H (refer to Table 2 for structures). Compounds 1H and 4H can be differentiated based on their mass spectra because they have different molecular weights. It was not possible to differentiate in this way between compounds 5H and 6H because they are isomers. Altering the ACN:H_2O ratio changed the retention times of the individual compounds. This ratio was changed to obtain optimal separation of the compounds as shown by the peaks in Figure 22. The ratio was incrementally changed between 90:10 and 75:25. It was concluded that the best resolution was obtained with water at the highest possible percentage so that compounds 5H and 6H were reasonably separated, and yet low enough so that the total separation was time efficient. More water in the mobile phase causes slower movement of the organic compounds through the

column. Conversely, higher concentrations of acetonitrile, an organic solvent, in the mobile phase moves the biphenylols more quickly through the column.

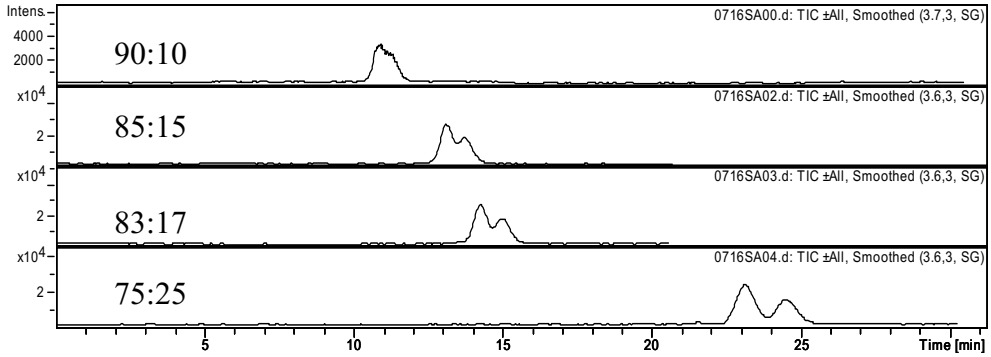


Figure 22: Separation of Compounds 5H and 6H

Peaks for all the experiments were identified based on the retention times that were determined from individual standards and molecular ion for each of the six compounds analyzed. It was necessary to use retention time for identification because compounds 1H, 2H, and 3H had the same molecular ion, 306, and compounds 4H, 5H, and 6H had the same molecular ion, 340 (refer to Table 2 for structures). Samples of each of the six stock solutions were analyzed at a ACN:H_2O ratio of 75:25 to determine their retention times. Table 12 shows the concentrations of the six compounds and their retention times, Figure 23.

Table 12: Retention Times for Each Compound

Compound	Conc in ACN (□g/mL)	Ret Time (min)
1H	5.0	14.25
2H	8.0	17.8
3H	4.0	19.96
4H	5.5	14.7
5H	6.2	24.2
6H	9.3	22.8

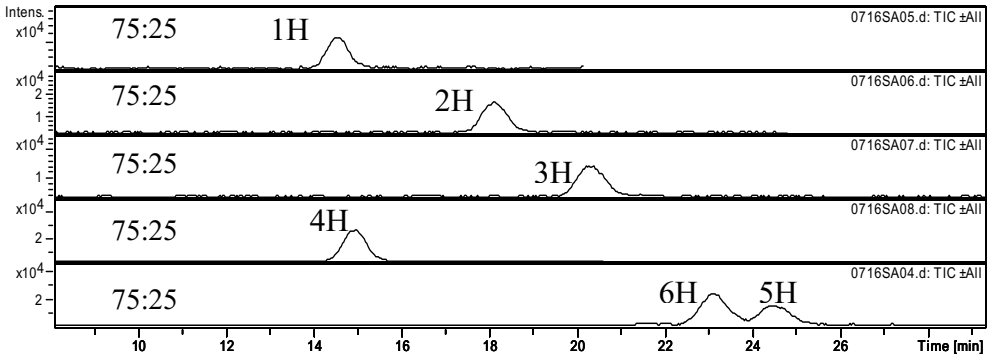


Figure 23: Chromatograms of Compounds 1H-6H Indicating Retention Times

The biphenylols could not be detected by the UV detector or on the plot from the MS in the positive ion mode. Because of the acidity of phenol groups, they can be analyzed as anions (28-31). Post-column base was added to deprotonate the biphenylols to give negative ions, which may be observed when the MS is in the negative ion mode. A 10:45:45 (NH_4OH:ACN:H_2O) solution was added by an external pump. The base was mixed with the effluent before it reached the detector. The flow rate of the base was altered from 0.2mL/min to 0.1mL/min. The flow rate of 0.1mL/min used for the analysis was adequate for detection and maintained the effluent at a moderately alkaline pH≈9.

The amount of solutions injected onto the column was varied from 10-200□L. Although a few peaks were visible at 10□L, more sample on the column facilitated detection. For reproducibility studies, the volume was increased to 100□L which was a full loop. The loop was changed in some experiments to a 200□L size loop in an attempt to increase detection with larger sample injections. The samples diluted with water and the SPE experiments were analyzed with a full 200□L loop to facilitate detection.

Mass Spectrometry

The mass spectrometric response was optimized by altering several variables of the analysis. The variables were types of compounds (methoxy and hydroxy PCBs), negative and positive modes, ion selection, and the amount of current applied at the electrospray interface.

A sample containing PCMBs was analyzed using the LC/ESI/MS instrument with the MS set in both positive and negative modes; however, no response was detected. It was apparent that the methoxy group on the PCMB could not be ionized by ESI and would not be detectable by LC/ESI/MS. The analytes of interest, OH-PCBs, were also analyzed with the MS set in positive and negative modes; however, no response was detected in the positive mode. The hydroxyl group on the OH-PCBs is ionizable with base and observable in the negative mode. This observation is in agreement with Sánchez-Rabaneda and Pérez-Magariño, who both report the necessity to study phenols in the negative mode (28-31). Thus the OH-PCBs were easily detected, but the PCMBs were not.

The separation of compounds 5H and 6H (refer to Table 2 for structures) was difficult to achieve on the LC column because the retention times overlapped. Co-eluting congeners may be identified by unique fragmentation profiles using GC/MS (32). It may be possible to use LC/ESI/MS in a similar manner to identify co-eluting congeners if different spectra can be achieved. To test this hypothesis, a sample containing only compounds 5H and 6H was introduced to the column. The current applied in the electrospray interface was increased to try to induce fragmentation in an attempt to achieve different fragmentation profiles. Unfortunately, no fragmentation was observed. Therefore, the separation was optimized using methods already described in the LC discussion.

The MS was set to scan for specific ions to achieve better signal to noise ratios for these compounds, thus gaining more sensitivity. The scan was set to select for masses 308.5 ±1.5 and 342.5 ±1.5 to detect major ions of the chlorine isotope clusters for the tetrachlorobiphenylols and pentachlorobiphenylols, respectively. The range of the scan was set to 3 amu to be sure the largest peaks in the chlorine isotope cluster could be observed. The entire isotope cluster of the deprotonated molecular ion of a tetrachlorobiphenylol analyzed in negative mode would include peaks at 305, 307, 309, 311, and 313, which are visible in an LC/MS scan from 280-400 m/z, see Figure 24. The entire isotope cluster of the deprotonated molecular ion of a pentachlorobiphenylol analyzed in negative mode includes peaks at 339, 341, 343, 345, 347, and 349, which are visible in an LC/MS scan from 280-400 m/z, see Figure 25.

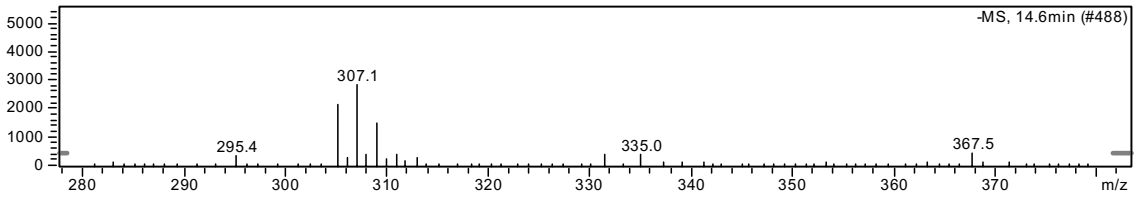


Figure 24: Compound 1H Complete LC/MS Spectrum

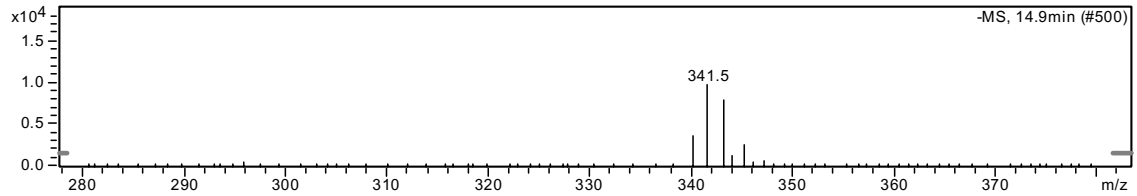


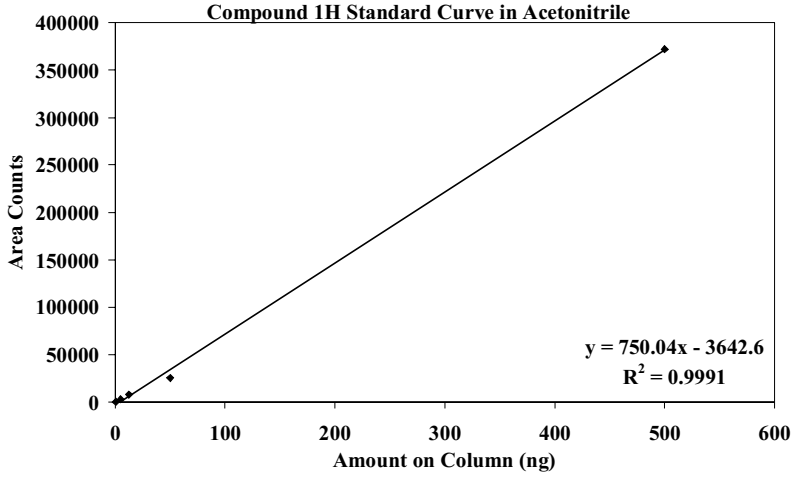
Figure 25: Compound 4H Complete LC/MS Spectrum

It was necessary to quantitate the response in area counts for each compound from the LC/ESI/MS data. The peak parameters had to be defined to calculate the area under each peak in a uniform manner. The baseline noise was manually determined. All peaks of the chromatograms were defined by assigning each peak a 1.5 minute window on the x-axis. This time designation was chosen after observing the elution profiles of all six OH-PCBs in several analyses. The average peak width at the baseline was 1.5 minutes. The area of each designated 1.5 minute section of the peaks was calculated above the determined noise levels by Bruker software using Sovitsky-Golay methods. The area counts were determined in this manner to be sure quantitation was performed uniformly for each analysis.

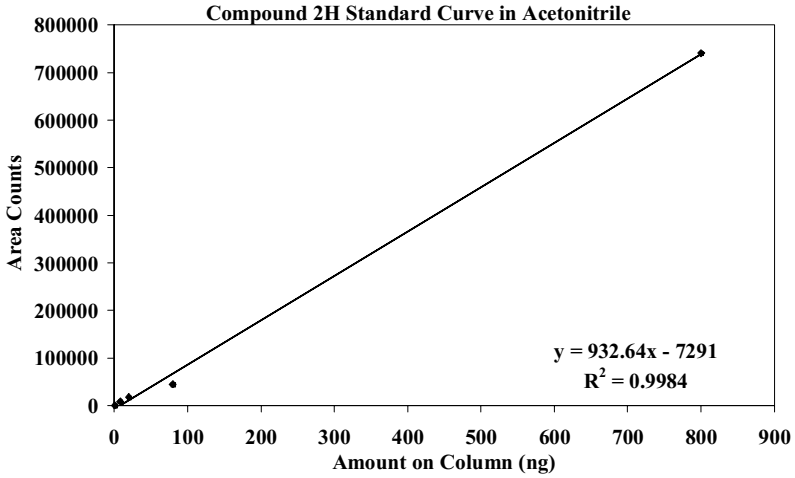
Standard Curve Experiments

The following standard curves, Figures 26-29, show a linear response in acetonitrile and in water. Comparison of the standard curves in water versus in acetonitrile indicates a lower response in acetonitrile.

A.



B.



C.

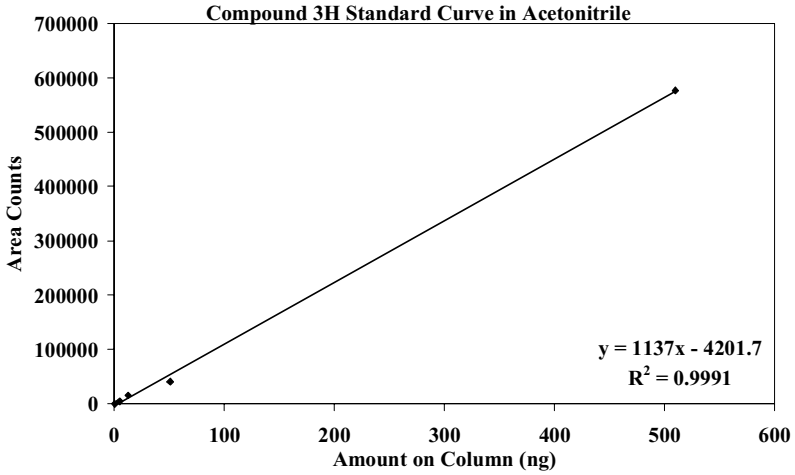
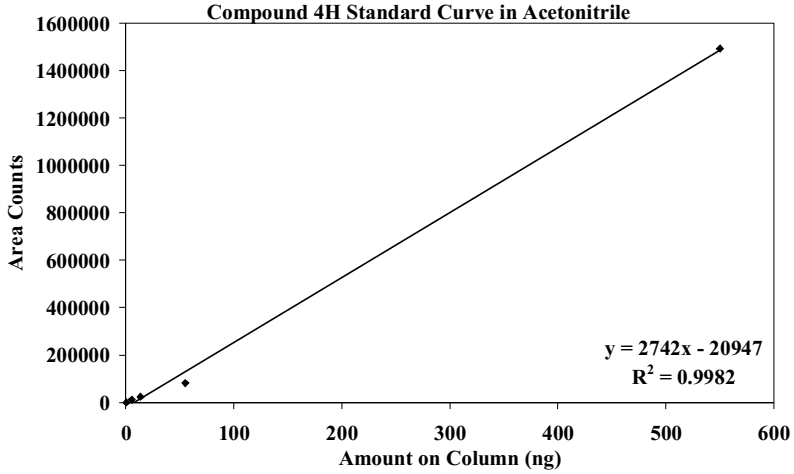
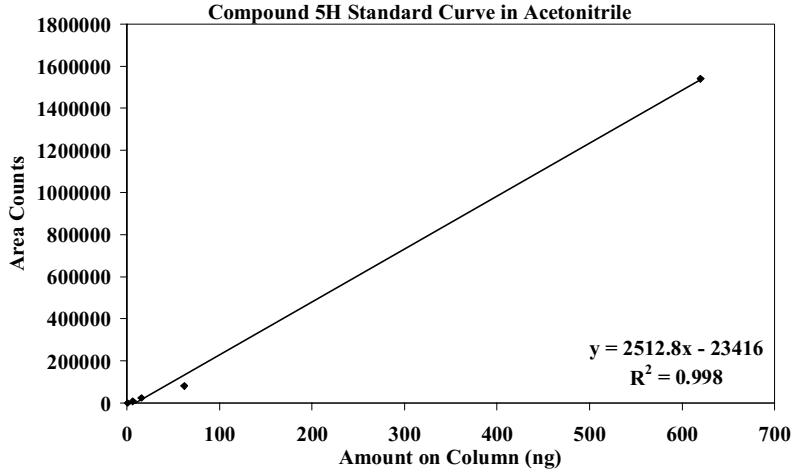


Figure 26: Standard Curves for Compounds 1H-3H in Acetonitrile

D.



E.



F.

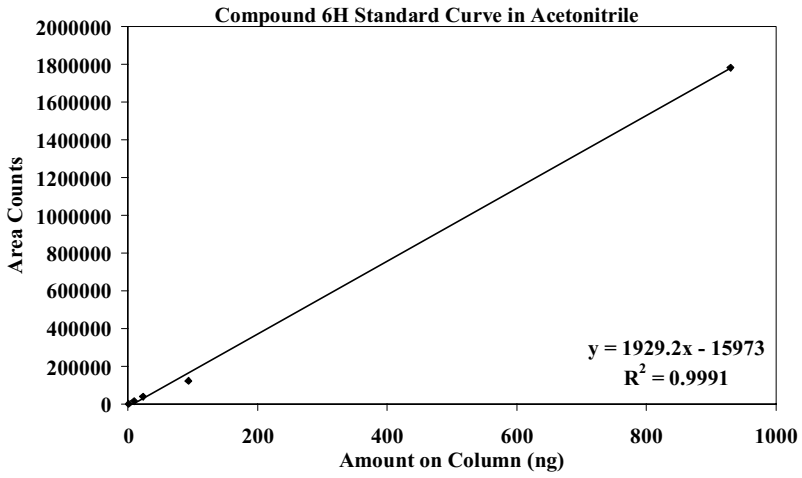
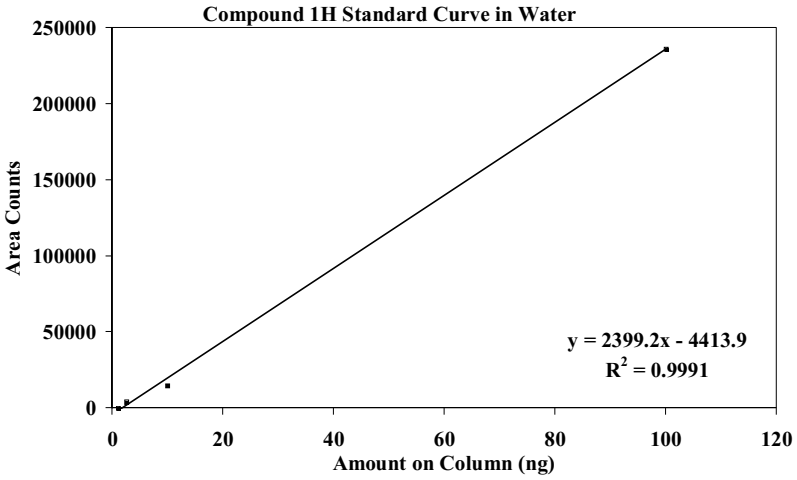
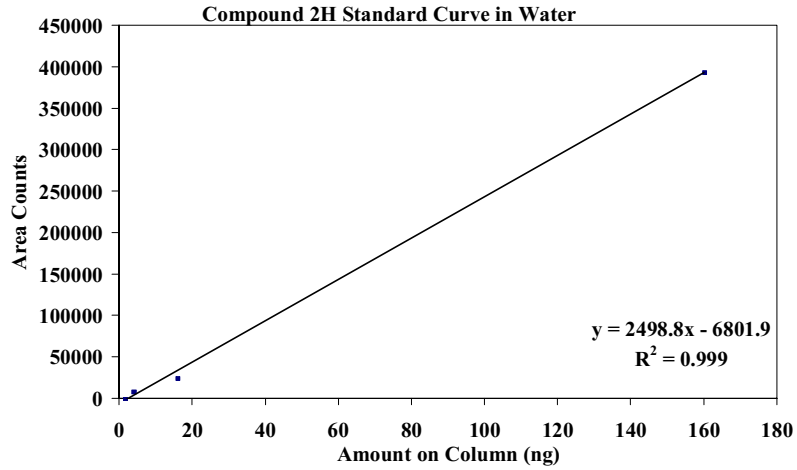


Figure 27: Standard Curves for Compounds 4H-6H in Acetonitrile

A.



B.



C.

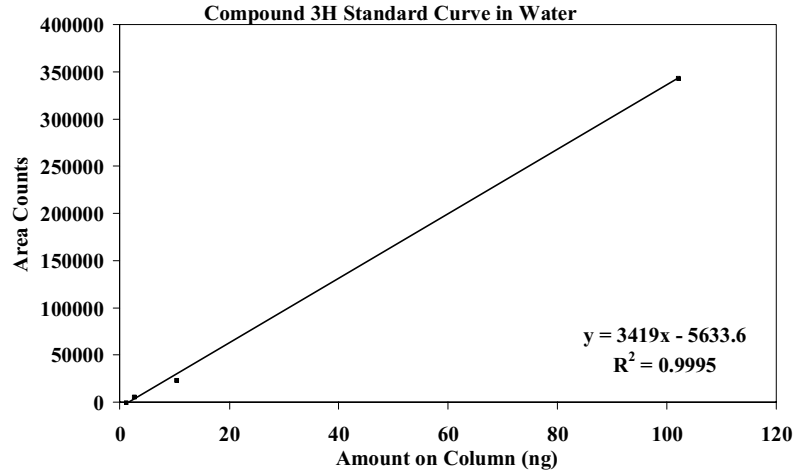
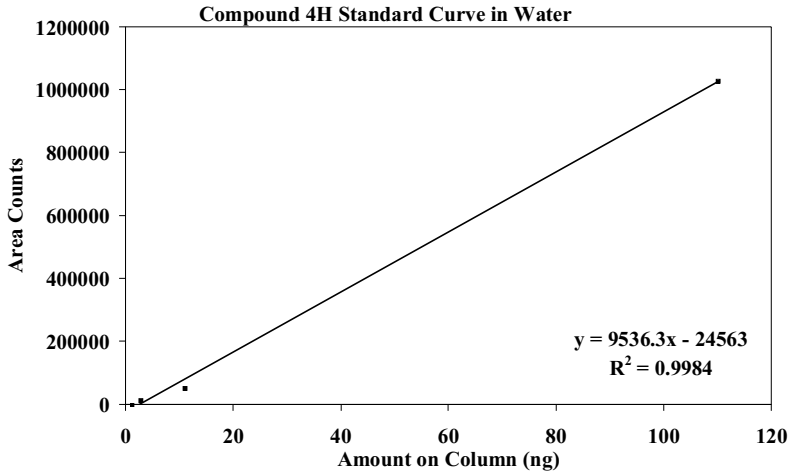
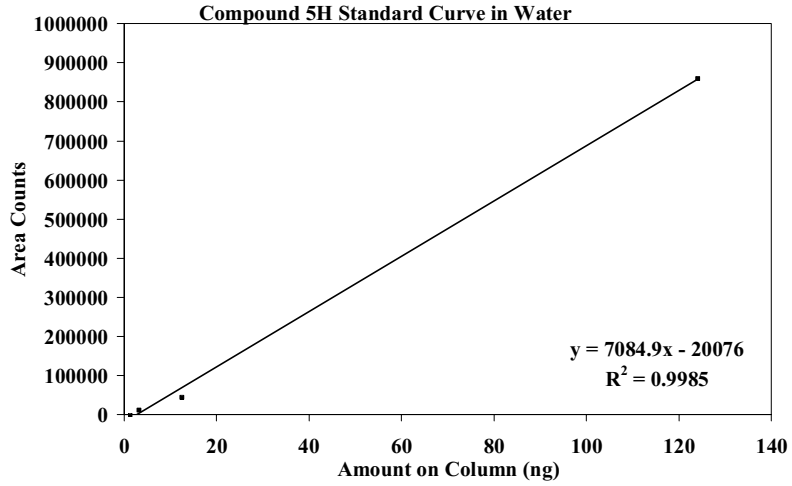


Figure 28: Standard Curves for Compounds 1H-3H in Water

D.



E.



F.

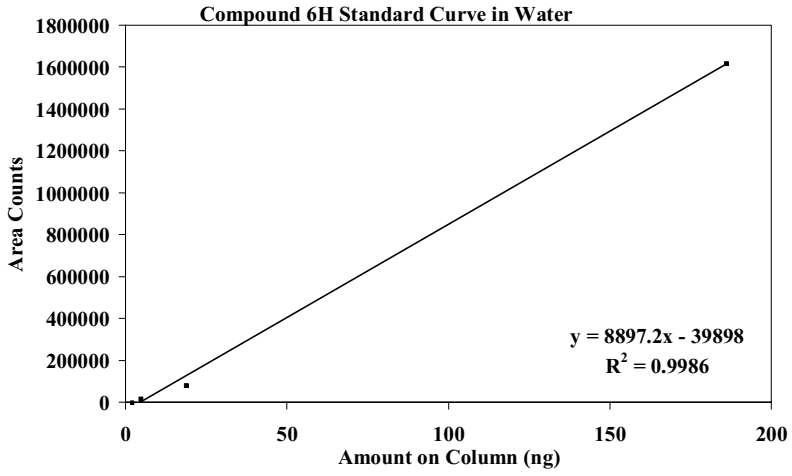


Figure 29: Standard Curves for Compounds 4H-6H in Water

Reproducibility Studies

To ensure reproducibility of the method, experiments were performed to analyze the precision of the LC/ESI/MS measurement using each of the six OH-PCB compounds (refer to Table 2 for structures) in both acetonitrile and water solutions where n=7. In Figures 30 and 31 it is evident that response is different for each compound per nanogram of material placed on the column. However, a general trend is that a higher response is seen for 94 percent and higher water solutions. This is advantageous because of the necessity to analyze these compounds in surface water samples. The error, given as percent standard deviation and shown as error bars for each compound in Figures 30-31, was calculated for each of these compounds in both acetonitrile and water. The error for estimating these compounds in water, ranging from 20-28%, is approximately the same as the error in acetonitrile, ranging from 11-19%. Another general trend can be seen of a higher response per nanogram of compound for the pentachlorinated OH-PCBs versus the response for tetrachlorinated OH-PCBs.

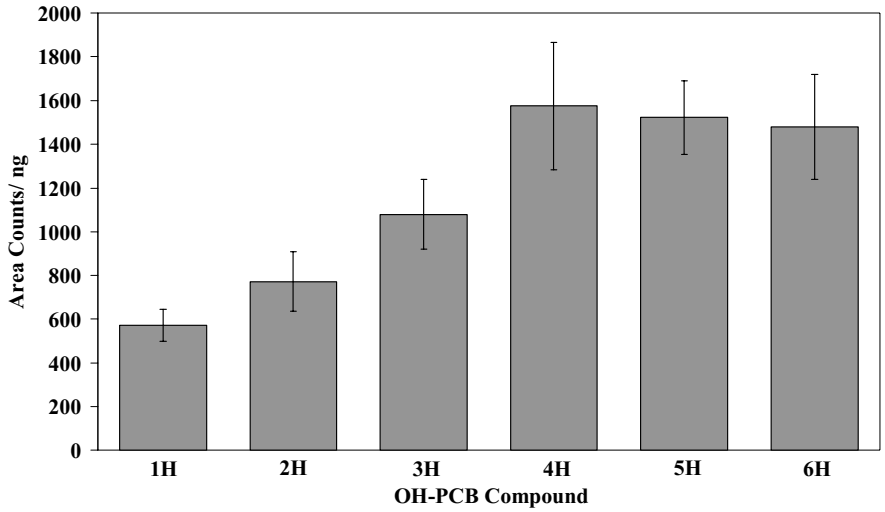


Figure 30: Reproducibility of Compounds 1H-6H in Acetonitrile

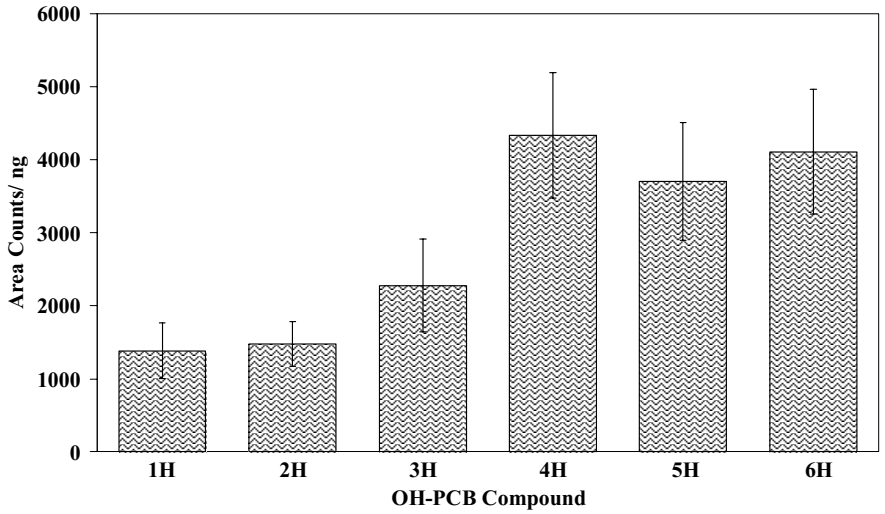


Figure 31: Reproducibility of Compounds 1H-6H in Water

Solid Phase Extraction Experiments

The initial experiments, 1 and 2, were performed with compound 4H (refer to Table 2 for structure) to determine the suitability of the SPE cartridges for analyte

isolation. Experiment 1 was performed specifically to test recovery of compound 4H when the compound was spiked into an aliquot of water on the column bed. Experiment 1 yielded the best result when compared to the standard data from the reproducibility study (see Figure 32). Experiment 2 was performed to test the effect on recovery of putting a large volume of water through the column which had been spiked with compound 4H (33). This experiment tests the ability of our system to detect compounds at a much lower concentration than was demonstrated in experiment 1, see Table 13. Experiment 3 is different from experiment 1 because the sample was concentrated by evaporation under a nitrogen flow after extraction from the column and then prepared for LC/MS analysis. The lower response suggests some loss of analyte in the concentration step. The concentration step was performed to try to enhance sensitivity, but may not be useful.

A control sample of distilled water with no spiked analytes was analyzed to confirm the absence of the analytes in the distilled water and on the SPE columns (see Figure C.7). There were no peaks for the OH-PCBs on the control chromatogram.

Recovery values of the analytes for SPE experiments 1-3 were determined from the standard curve data for compound 4H (see Figure 29, D). The column labeled "Calculated Area Counts" in Table 13 is calculated based on the equation from the regression analysis of the standard curve data for compound 4H in water. Experiments 1 and 2 have very similar recovery even though experiment 2 represents a sample that is much less concentrated. The low recovery for experiment 3 can be attributed to the concentration step, as previously mentioned.

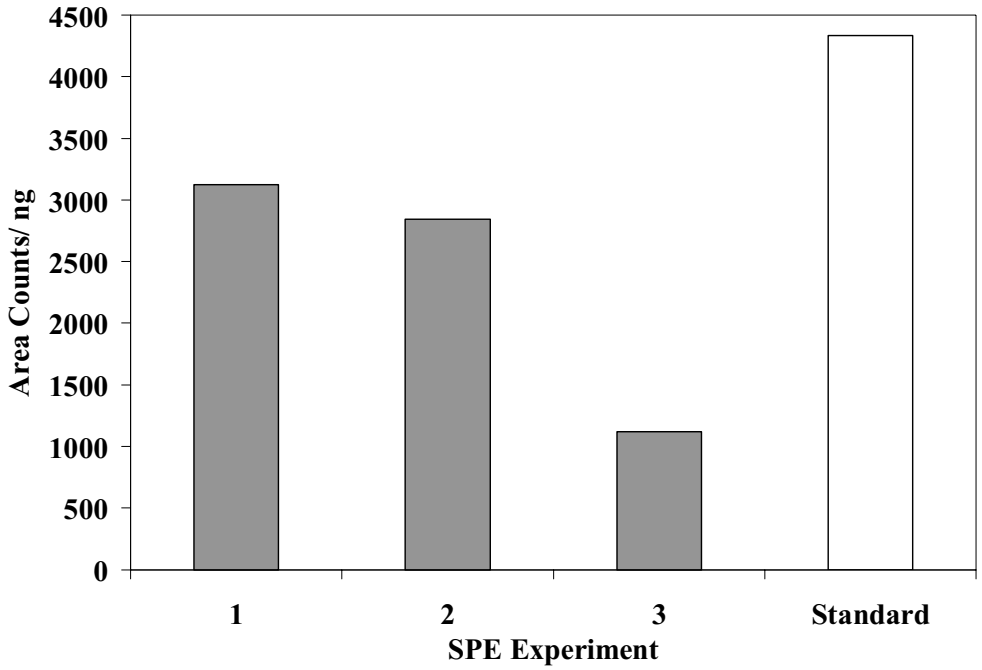


Figure 32: SPE Experiments 1-3: Spiked Cartridge with Compound 4H

Table 13: Recovery for Compound 4H in SPE Experiments 1-3

Experiment	Compound	Concentration (ng/mL=ppb)	Amount on Column (ng)	Area Counts	Calculated Area Counts	% Recovery
1	4H	55	11	34344	80336.30	42.75
2	4H	4.4	11	31316	80336.30	38.98
3	4H	5.5	22	24632	185235.60	13.30

Experiment 4-1 is different from experiment 3 because, in the former experiment, a sample of water spiked with the compound was placed on the cartridge, rather than spiking an aliquot on the cartridge and washing with water. These data indicate a similar response for experiment 4-1 and experiment 3 for all of the analytes, suggesting that the method used in experiment 4-1 does not alter the recovery of the analytes. It is important to test the method in experiment 4-1 because this technique would be used for an actual

water sample. It would be necessary to pass the water sample through the cartridge and elute the compound from the cartridge for LC/MS analysis. This was done in the surface water experiments described below. It is noteworthy to mention that the best results might be obtained if the sample were not concentrated after extraction from the column, as in experiments 1 and 2.

Recovery values of the analytes for SPE experiments 3 and 4-1 were determined from the standard curve data for the six OH-PCBs (see Figures 28 and 29). The columns labeled "Calculated Area Counts" in Tables 14 and 15 are calculated based on regression analysis of the standard curve data for each of the six OH-PCBs in water. There is no significant difference in recovery for experiment 4-1 compared to experiment 3, despite the lower sample concentrations in experiment 4-1. This indicates that the method used in experiment 4-1 is practical for surface water experiments.

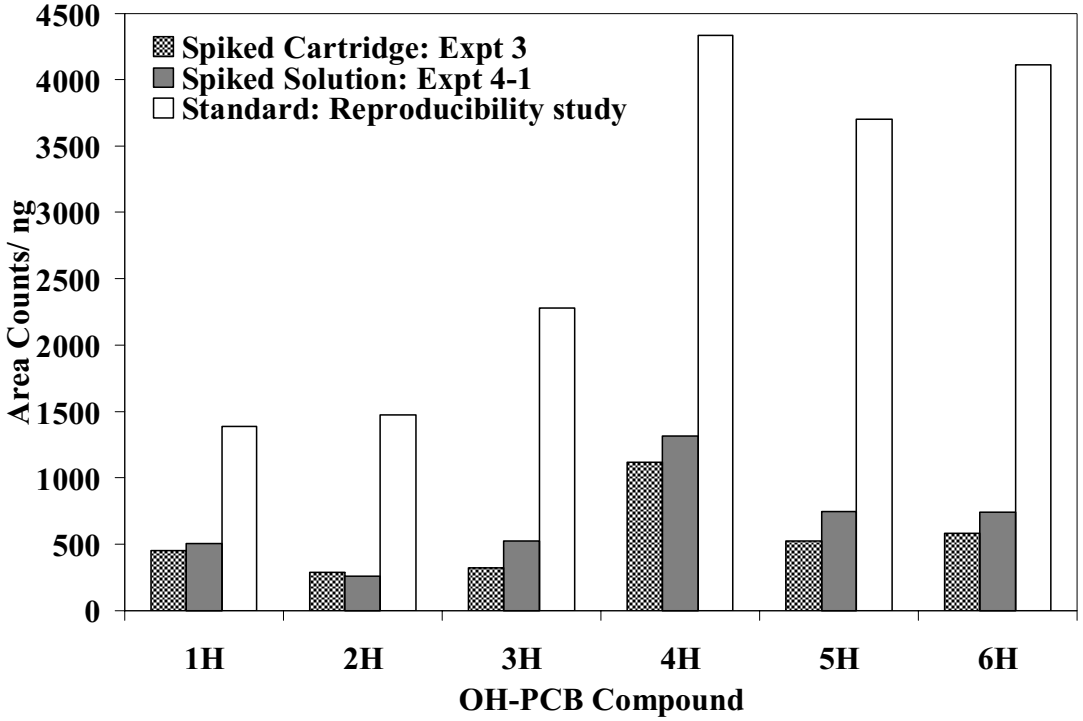


Figure 33: SPE Experiment 3 and 4-1: Spiked Cartridge vs Spiked Solution

Table 14: Recovery for Six OH-PCBs in SPE Experiment 3

Compound	Concentration (ng/mL=ppb)	Amount on Column (ng)	Area Counts	Calculated Area Counts	% Recovery
1H	5	20	9064	43570.10	20.80
2H	8	32	9210	73159.70	12.59
3H	5.1	20.4	6577	64114.00	10.26
4H	5.5	22	24632	185235.60	13.30
5H	6.2	24.8	13072	155629.52	8.40
6H	9.3	37.2	21684	291077.84	7.45

Table 15: Recovery for Six OH-PCBs in SPE Experiment 4-1

Compound	Concentration (ng/mL=ppb)	Amount on Column (ng)	Area Counts	Calculated Area Counts	% Recovery
1H	2	20	10051	43570.10	23.07
2H	4	32	8314	73159.70	11.36
3H	2.04	20.4	10653	64114.00	16.62
4H	2.2	22	28904	185235.60	15.60
5H	2.48	24.8	18546	155629.52	11.92
6H	3.72	37.2	27597	291077.84	9.48

Experiment 4-2 was performed to determine if the compounds were being adsorbed onto the glass vials used to prepare the sample. It appears that there is no significant difference in the response for the tetrachloro-biphenylols. However, the pentachloro-biphenylols show lower levels with glass compared to plastic. This difference was not considered significant enough to change the method of sample preparation for this study.

Recovery values of the analytes for SPE experiments 4-2 were determined from the standard curve data for the six OH-PCBs (see Figures 28 and 29). The column labeled “Calculated Area Counts” in Table 16 is calculated based on regression analysis of the standard curve data for each of the six OH-PCBs in water. There is no significant difference in recovery for compounds 1H-6H from experiments 4-1 to 4-2.

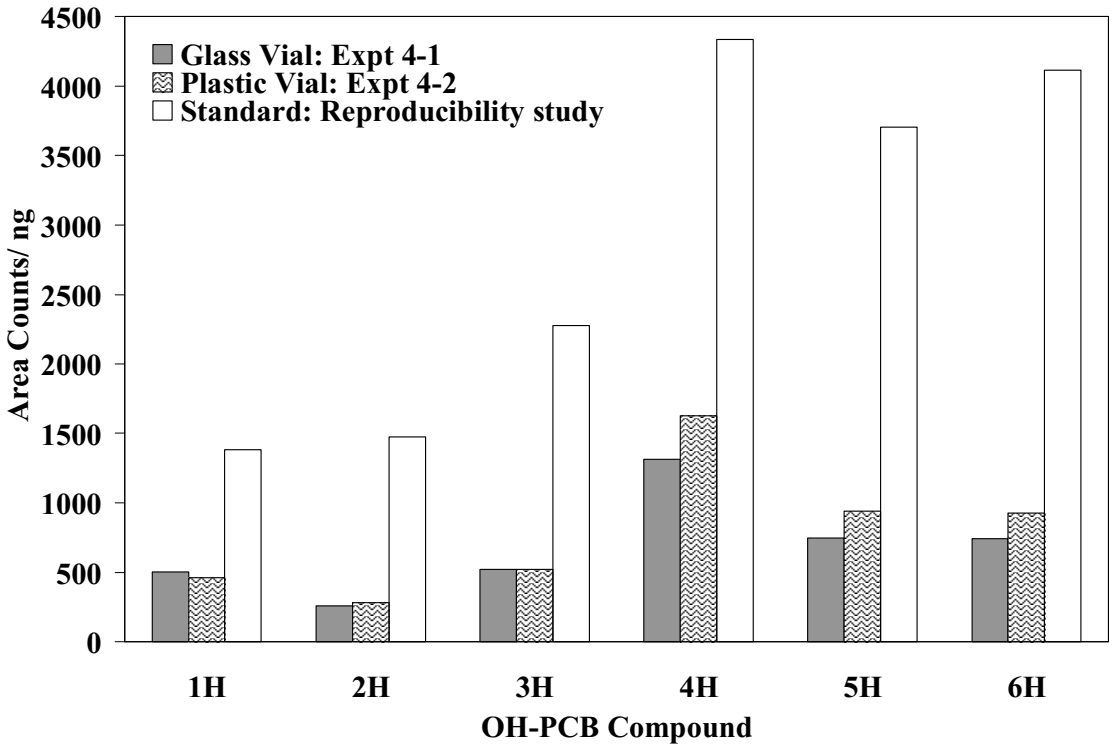


Figure 34: SPE Experiment 4-1 and 4-2: Glass vs Plastic Vial

Table 16: Recovery for Six OH-PCBs in SPE Experiment 4-2

Compound	Concentration (ng/mL=ppb)	Amount on Column (ng)	Area Counts	Calculated Area Counts	% Recovery
1H	2	20	9254	43570.10	21.24
2H	4	32	9105	73159.70	12.45
3H	2.04	20.4	10608	64114.00	16.55
4H	2.2	22	35768	185235.60	19.31
5H	2.48	24.8	23352	155629.52	15.00
6H	3.72	37.2	34426	291077.84	11.83

Experiments 5-1 and 5-2 were performed to determine levels of these compounds in surface water samples. Both samples were prepared by spiking an aliquot of the surface water and then filtering the aliquot before placing the samples on the SPE

column. The filtering process was used to clean up the sample before extraction and analysis. Clearly, experiment 5-1 yielded higher recoveries than experiment 5-2.

Control samples with the two types of surface water with no spiked analytes were filtered and analyzed to confirm the absence of the analytes in question in the surface water samples (see Figure C.9). There were no peaks for the OH-PCBs on the control chromatograms.

Recovery values of the analytes for SPE experiments 5-1 and 5-2 were determined from the standard curve data for the six OH-PCBs (see Figures 28 and 29). The columns labeled "Calculated Area Counts" in Tables 17 and 18 were calculated based on regression analysis of the standard curve data for each of the six OH-PCBs in water. There is a significant difference in recovery values for experiments 5-1 and 5-2, which can be attributed to possible binding of the analytes by the biological material in the surface sample used in experiment 5-2. Interestingly, recovery for the surface water samples is higher than recovery for the distilled water samples. This is promising for possible future applications to real-world surface water testing.

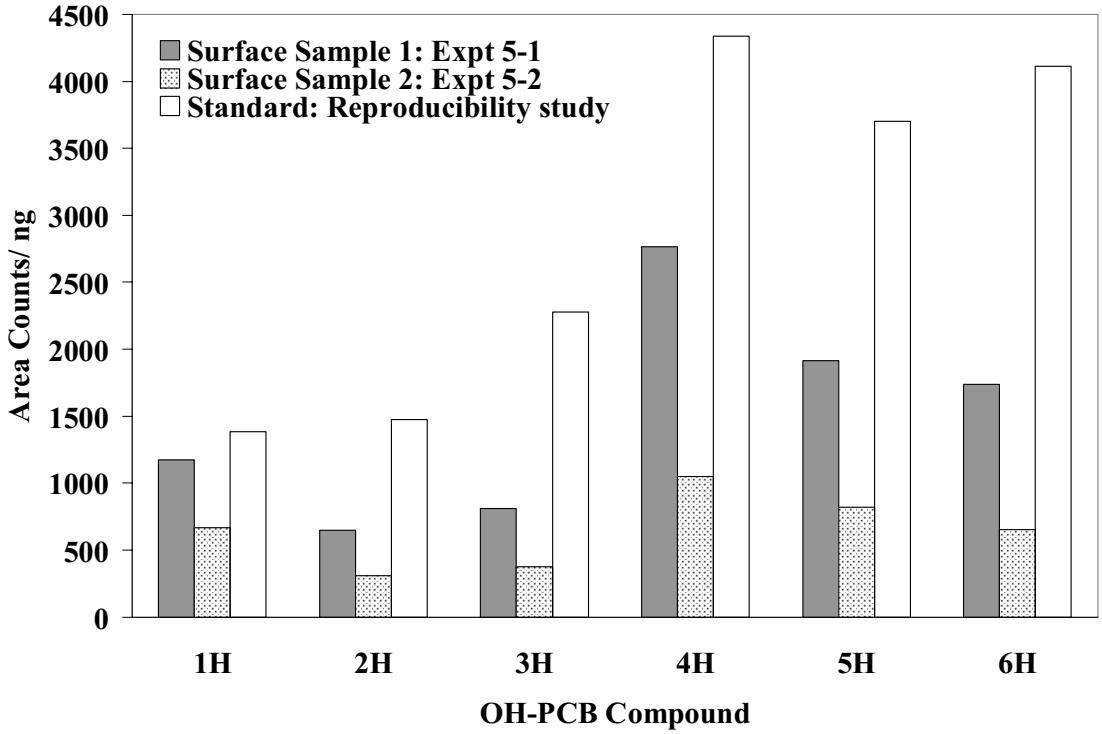


Figure 35: SPE Experiment 5-1 and 5-2: Surface Water Studies

Table 17: Recovery for Six OH-PCBs in SPE Experiment 5-1

Compound	Concentration (ng/mL=ppb)	Amount on Column (ng)	Area Counts	Calculated Area Counts	% Recovery
1H	2	20	23456	43570.10	53.84
2H	4	32	20700	73159.70	28.29
3H	2.04	20.4	16536	64114.00	25.79
4H	2.2	22	60875	185235.60	32.86
5H	2.48	24.8	47521	155629.52	30.53
6H	3.72	37.2	64629	291077.84	22.20

Table 18: Recovery for Six OH-PCBs in SPE Experiment 5-2

Compound	Concentration (ng/mL=ppb)	Amount on Column (ng)	Area Counts	Calculated Area Counts	% Recovery
1H	2	20	13355	43570.10	30.65
2H	4	32	9978	73159.70	13.64
3H	2.04	20.4	7698	64114.00	12.01
4H	2.2	22	23084	185235.60	12.46
5H	2.48	24.8	20320	155629.52	13.06
6H	3.72	37.2	24316	291077.84	8.35

It is possible that the particulates and biological material in the surface water samples were binding the analytes. This process would cause the analytes to be removed from solution during the filtration step. The particulate binding experiment was performed with surface water sample 5-1 because it was a natural body of flowing water with relatively little plant material. The differences seen in the filtered versus unfiltered sample 5-1 may be caused by the loss of some analytes during the filtration of the sample.

Recovery values of the analytes for SPE experiments 5-3 were determined from the standard curve data for the six OH-PCBs (see Figures 28 and 29). The column labeled “Calculated Area Counts” in Table 19 was calculated based on regression analysis of the standard curve data for each of the six OH-PCBs in water. The difference in recovery between the filtered an unfiltered sample is more significant than other changes to the method. This indicates that the filtration step may be responsible for some loss of analytes. The best recovery would be obtained by not filtering the sample.

Recovery from surface water sample 5-2 was much lower than that of sample 5-1, and it could be assumed that particulate binding occurred in that sample as well, and some of the analytes were filtered out before going through the SPE column. However, an analysis without the filtration step was not performed for sample 5-2 to confirm this assumption.

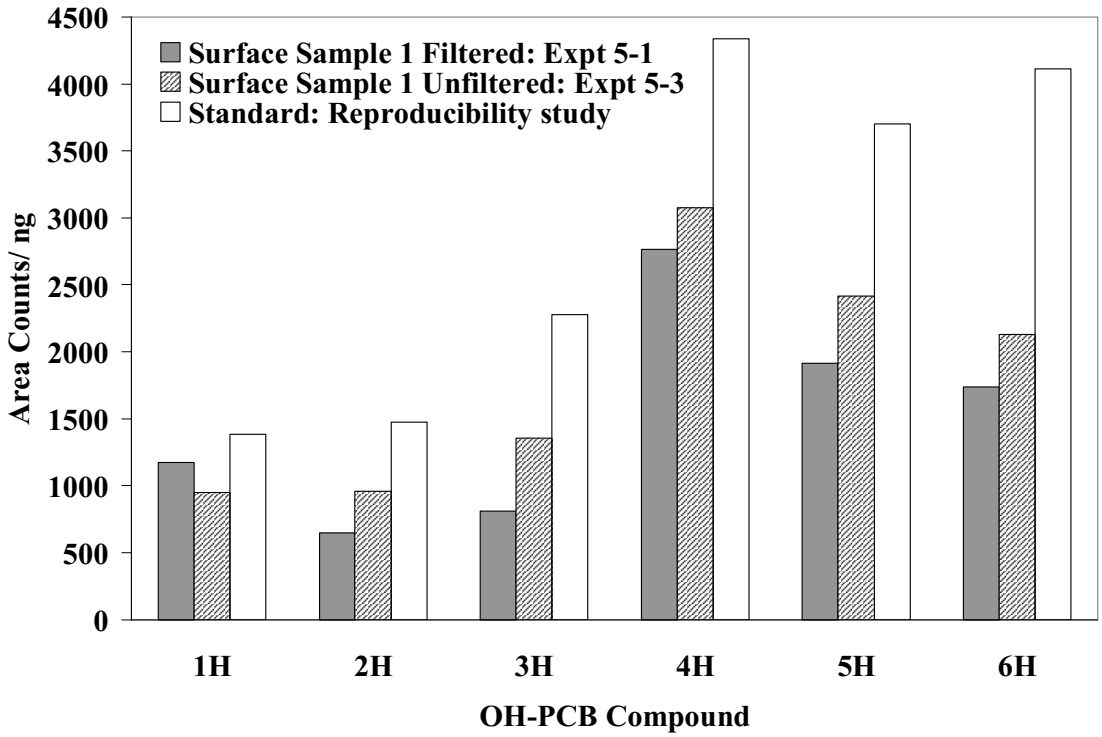


Figure 36: SPE Experiment 5-1 and 5-3: Particulate Binding

Table 19: Recovery for Six OH-PCBs in SPE Experiment 5-3

Compound	Concentration (ng/mL=ppb)	Amount on Column (ng)	Area Counts	Calculated Area Counts	% Recovery
1H	2	20	19004	43570.10	43.62
2H	4	32	30745	73159.70	42.02
3H	2.04	20.4	27662	64114.00	43.15
4H	2.2	22	67675	185235.60	36.53
5H	2.48	24.8	59865	155629.52	38.47
6H	3.72	37.2	79166	291077.84	27.20

Detection Limits

For determination of detection limits, the six OH-PCBs were analyzed individually. Signal to noise was determined by comparing the area counts for the integrated peak of each compound with the area counts for an integrated 1.5 minute section of the baseline. Table 20 includes data for each experiment in terms of analyte mass on the column and S/N ratios for the reproducibility studies and the SPE experiments 4-1, 4-2, 5-1, 5-2, and 5-3 (refer to Tables 4, 5, and 21 for concentrations). Many of the data correspond to the limit of detection where the S/N≈3. The lowest concentration of analyte detected by this SPE-LC/ESI/MS method was 2ng/mL (2 ppb) of compound 1H in experiments 4-1, 4-2, 5-1, 5-2, and 5-3. This concentration for compound 1H was obtained by diluting 10□L of 5□g/mL of compound 1H (dilution 1 in acetonitrile, see Table 4) to 25mL with distilled water. The spiked sample contained 50ng of compound 1H. The 25mL of sample solution was passed through the SPE tube. The compound was eluted from the tube with 5mL of acetonitrile. The eluent was concentrated to approximately 200□L and diluted to 500□L with water and analyzed with 200□L injections containing 20ng of compound 1H. Similar concentrations were

detected for compound 2H-6H, approximately 2-4ng/mL. Therefore, the detection limit in water for the SPE experiments was determined to be 2-4ppb.

Table 20: S/N Values for OH-PCBs in Reproducibility Studies and SPE Experiments

	OH-PCB Compound					
	1H		2H		3H	
Experiment	S/N	Amt on Column (ng)	S/N	Amt on Column (ng)	S/N	Amt on Column (ng)
Reproduc. ACN	1.4/1	12.5	3.2/1	20	2.8/1	12.8
Reproduc. H2O	2.0/1	10	3.3/1	16	3.2/1	10.2
4-1	2.7/1	20	2.2/1	32	2.9/1	20.4
4-2	1/1	20	1/1	32	1.2/1	20.4
5-1	4.6/1	20	4/1	32	3.2/1	20.4
5-2	2.7/1	20	2/1	32	1.6/1	20.4
5-3	1.8/1	20	2.9/1	32	2.6/1	20.4
	4H		5H		6H	
Experiment	S/N	Amt on Column (ng)	S/N	Amt on Column (ng)	S/N	Amt on Column (ng)
Reproduc. ACN	3.1/1	13.8	3.1/1	15.5	5.0/1	23.3
Reproduc. H2O	5.2/1	11	4.5/1	12.4	8.4/1	18.6
4-1	5.6/1	22	3.6/1	24.8	5.3/1	37.2
4-2	3.4/1	22	2.2/1	24.8	3.2/1	37.2
5-1	13.5/1	22	10.5/1	24.8	14.3/1	37.2
5-2	3.3/1	22	2.9/1	24.8	3.5/1	37.2
5-3	6.7/1	22	6.0/1	24.8	7.9/1	37.2

Table 21: Concentrations of OH-PCBs in SPE Experiments

	OH-PCB Concentration (ng/mL)					
Experiment	1H	2H	3H	4H	5H	6H
4-1	2	4	2.04	2.2	2.48	3.72
4-2	2	4	2.04	2.2	2.48	3.72
5-1	2	4	2.04	2.2	2.48	3.72
5-2	2	4	2.04	2.2	2.48	3.72
5-3	2	4	2.04	2.2	2.48	3.72

CHAPTER VI

CONCLUSION

Six PCMBs were successfully synthesized using the Suzuki coupling method. The corresponding OH-PCBs were successfully synthesized by demethylating the PCMBs. The PCMB structures were confirmed by GC/MS, IR, and NMR data. The OH-PCB structures were confirmed by GC/MS.

A method using SPE coupled with LC/ESI/MS was tested for detection of OH-PCBs in distilled and surface water samples. The detection limit in water for the SPE experiments was determined to be 2-4ppb. This amount is reasonable for expected limits observed in environmental water samples. Current GC/MS analytical methods for PCB detection in water samples generally detect at low □g/L or ppb, with some limits down to ppt (35).

It was determined that the best recovery of analytes using the SPE column might be obtained by using no filtration step, if possible, and not concentrating the eluent before injecting the sample in the LC/ESI/MS. The optimal LC conditions are dependent on the column used and the solvent system. A solvent system with 75% acetonitrile and 25% water was determined to provide optimal separation of the six OH-PCBs with a flow rate of 0.9mL/min. A post column infusion of base solution of 10% ammonium hydroxide was used to enhance anion formation. Optimal MS conditions include scanning in negative mode and using ion selectivity if the molecular ions are known.

This method is satisfactory for detection of these analytes in surface water and should be useful for detecting other tetrachloro and pentachlorobiphenylols isomers under the same conditions. Use of this method alone would not allow for the specific assignment of structures of unknown analytes. Further research is necessary for this method to be more quantitative for unknown samples. The conditions mentioned in the SPE experiments would need to be optimized for reproducibility and sensitivity.

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APPENDIX A

GAS CHROMATOGRAPHY/ MASS SPECTROMETRY DATA

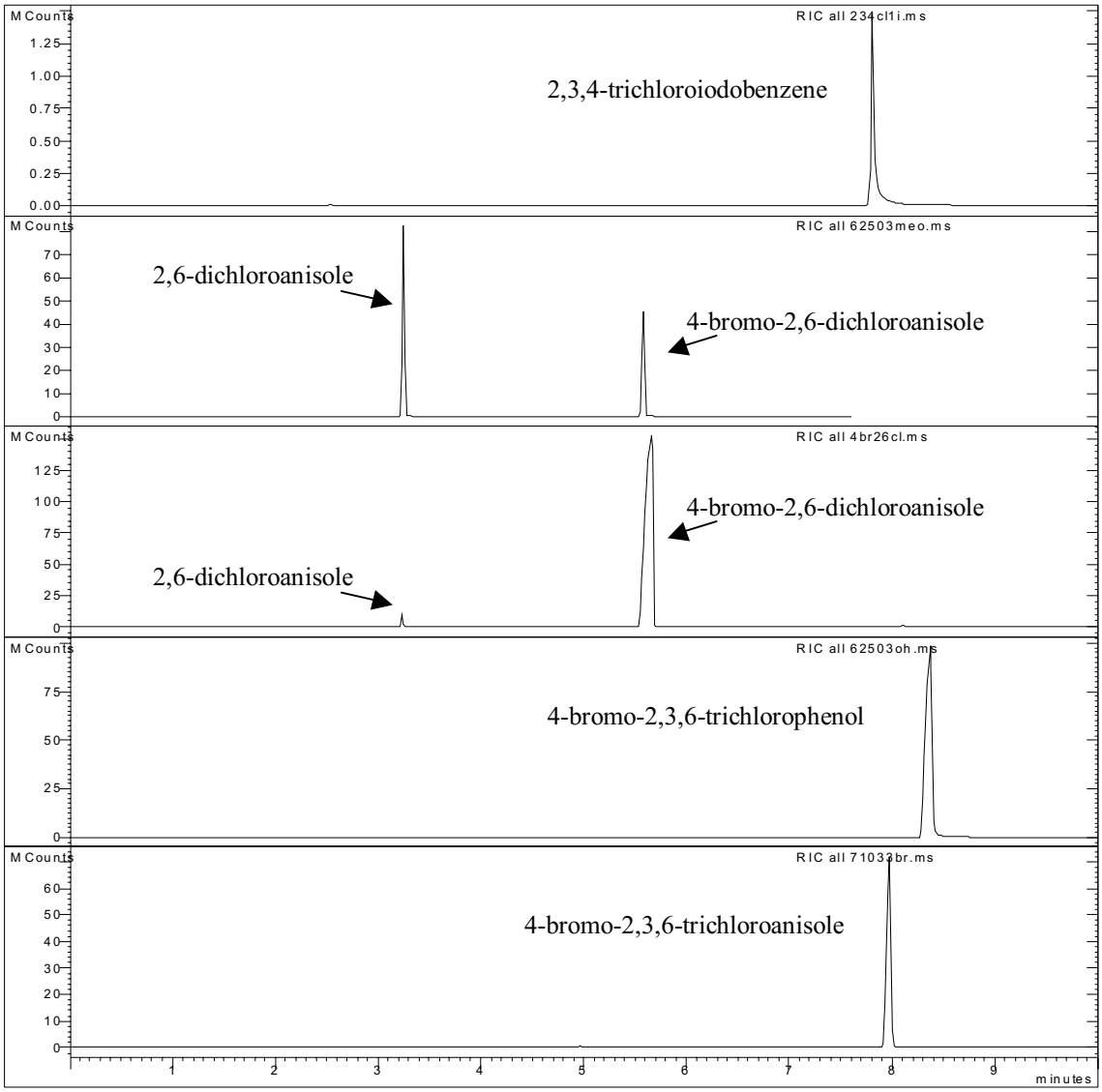

Figure A.1: GC Chromatograms of Materials Used in Preparation of Methoxypolychlorobiphenyls

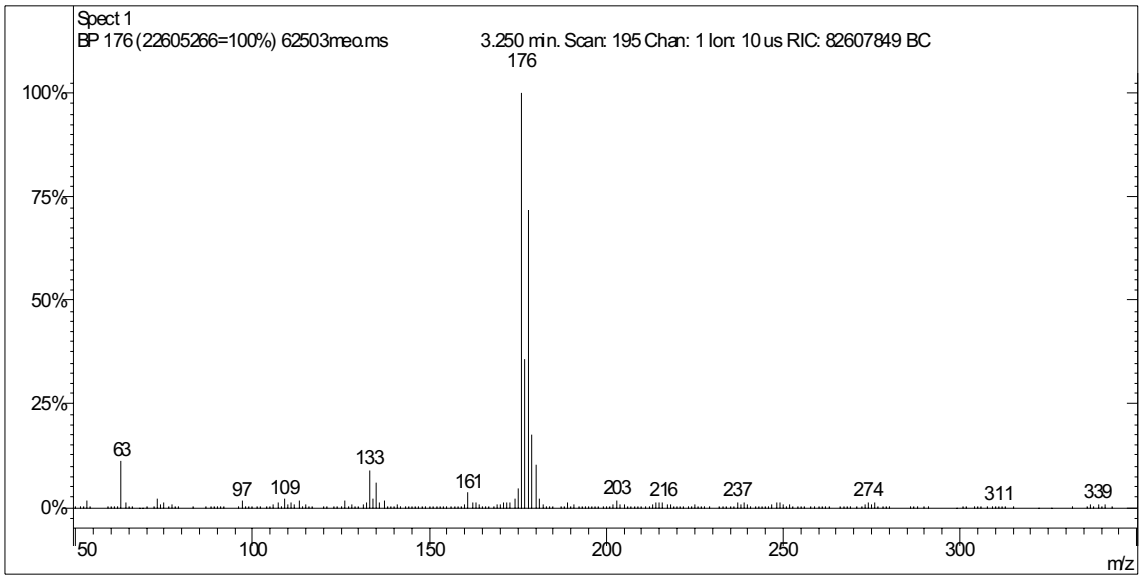

Figure A.2: 2,6-dichloroanisole GC/ Mass Spectrum

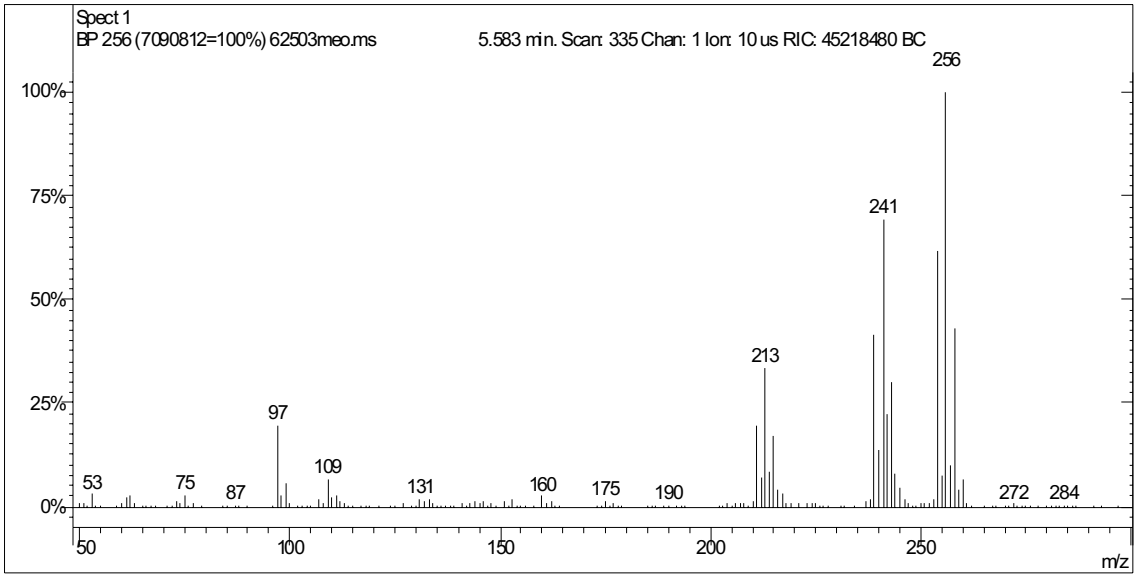

Figure A.3: 4-bromo-2,6-dichloroanisole GC/ Mass Spectrum

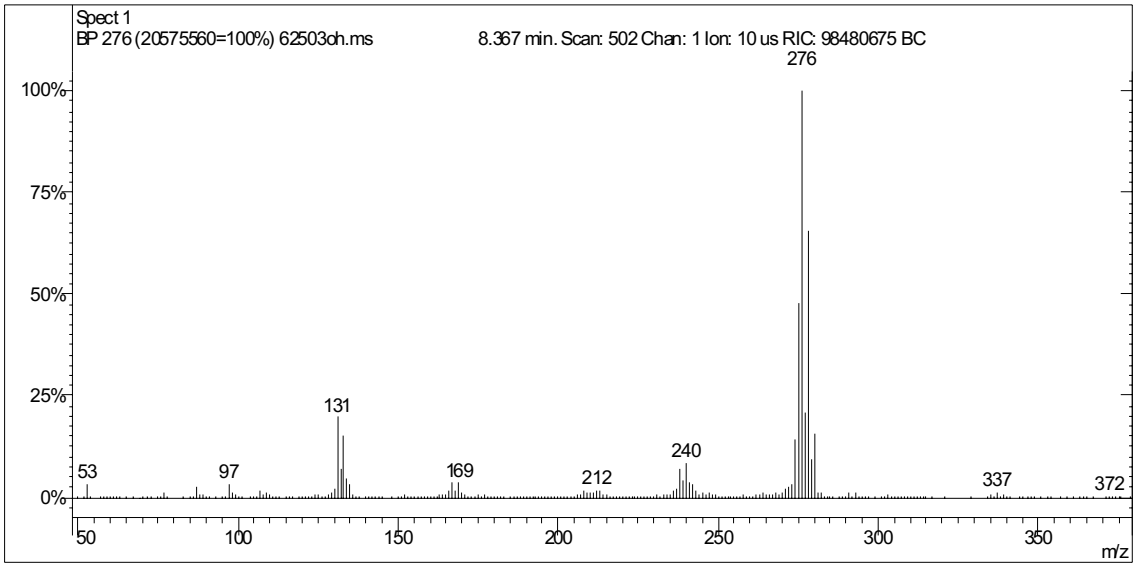

Figure A.4: 4-bromo-2,3,6-trichlorophenol GC/ Mass Spectrum

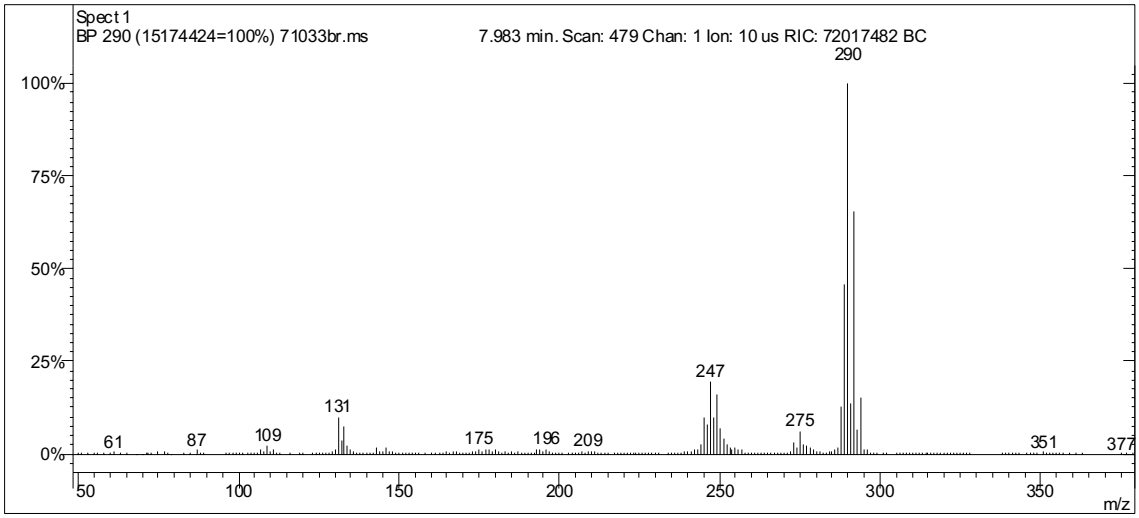

Figure A.5: 4- bromo-2,3,6-trichloroanisole Mass Spectrum

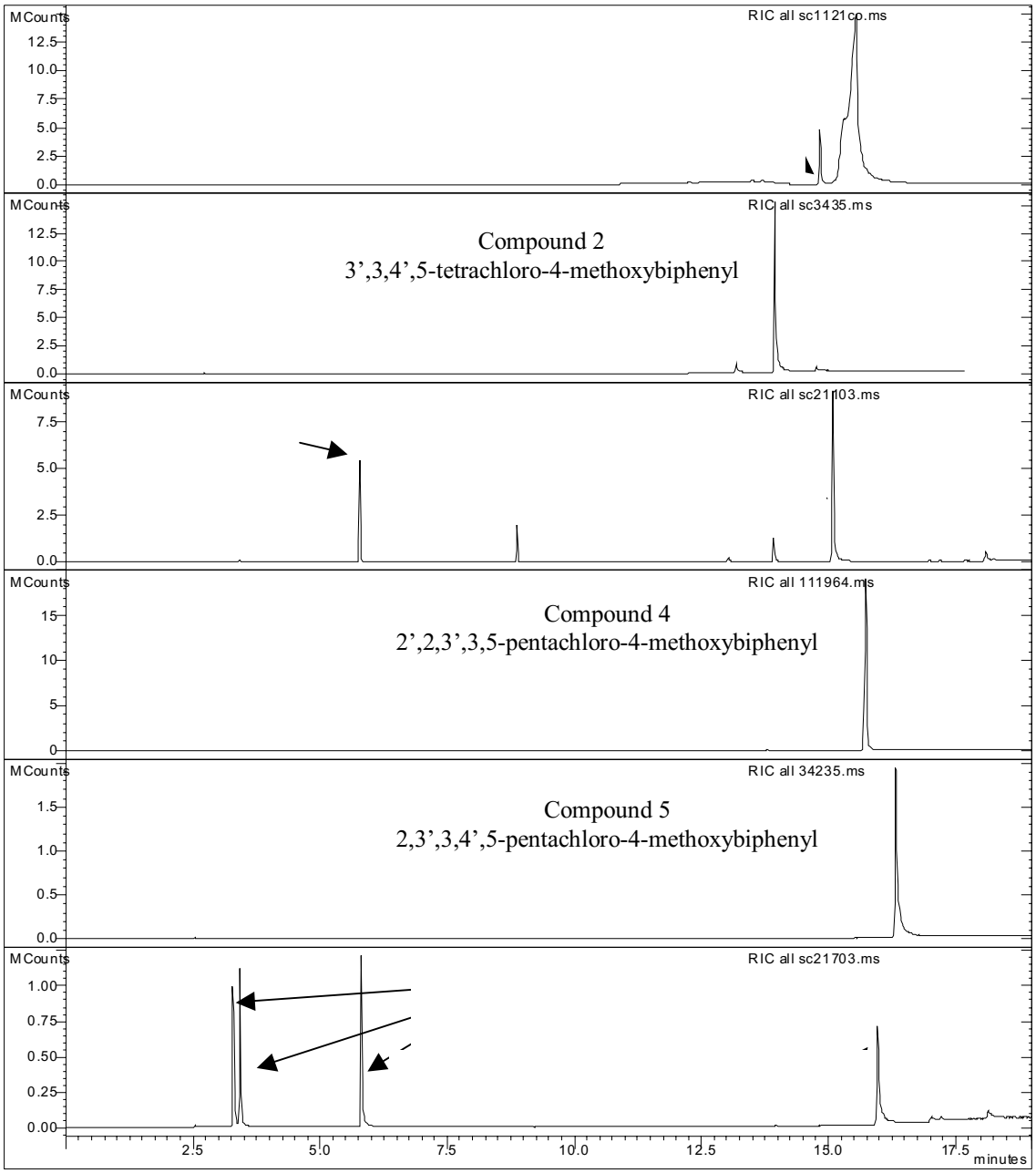

Figure A.6: GC Chromatograms of Methoxypolychlorobiphenyls 1-6

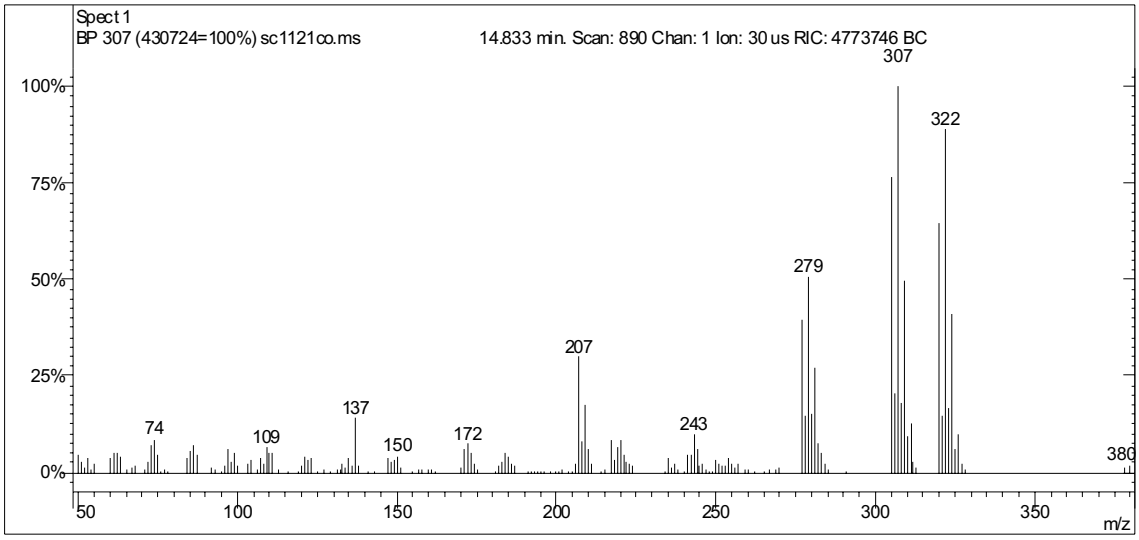

Figure A.7: 2',3',3,5-tetrachloro-4-methoxybiphenyl GC/ Mass Spectrum

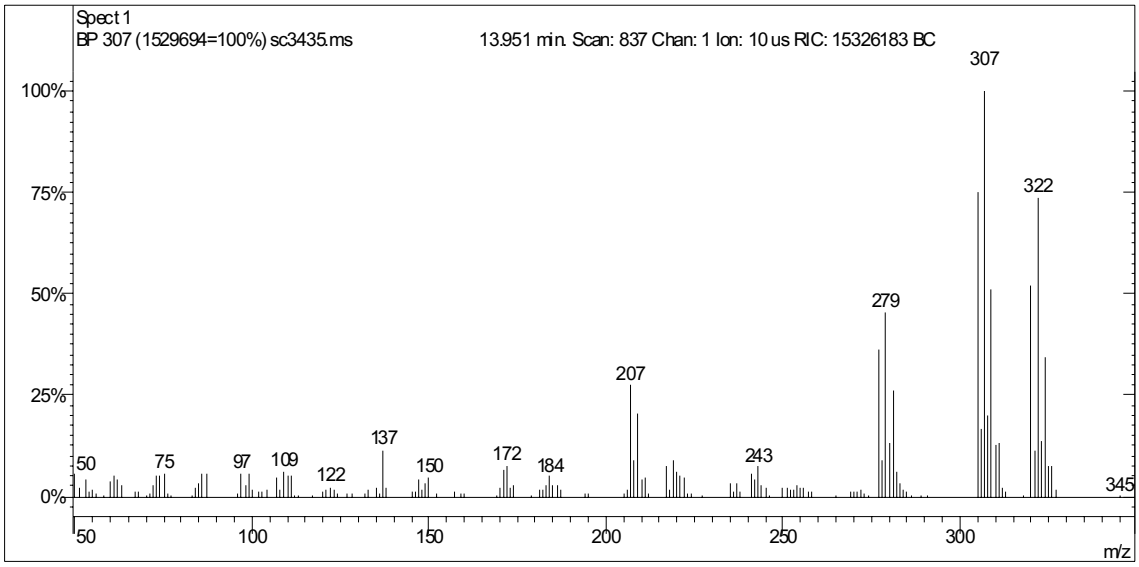

Figure A.8: 3',3,4',5-tetrachloro-4-methoxybiphenyl GC/ Mass Spectrum

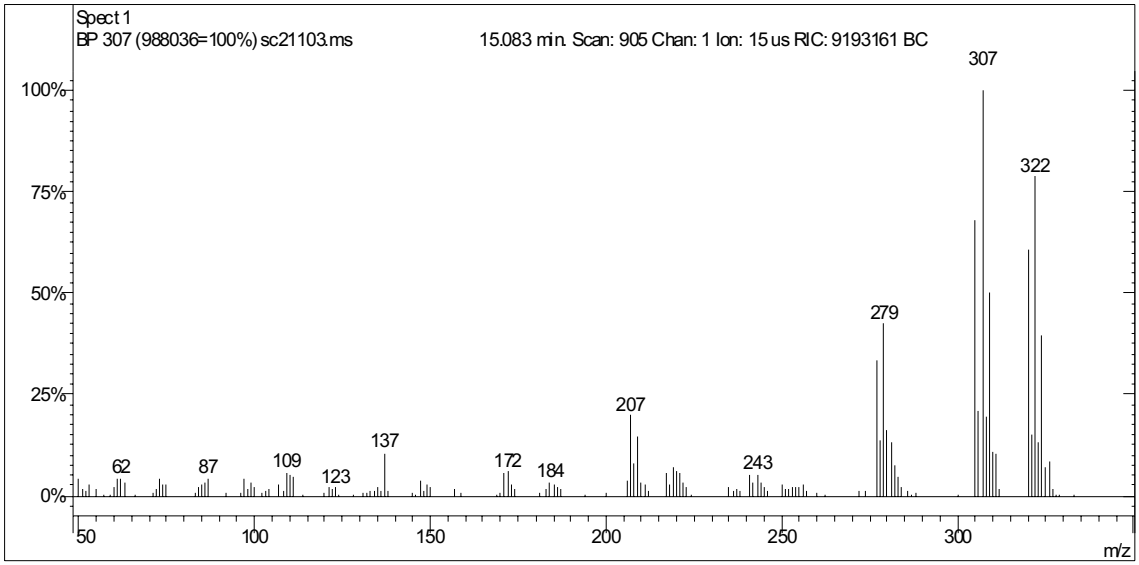

Figure A.9: 3',3,5',5-tetrachloro-4-methoxybiphenyl GC/ Mass Spectrum

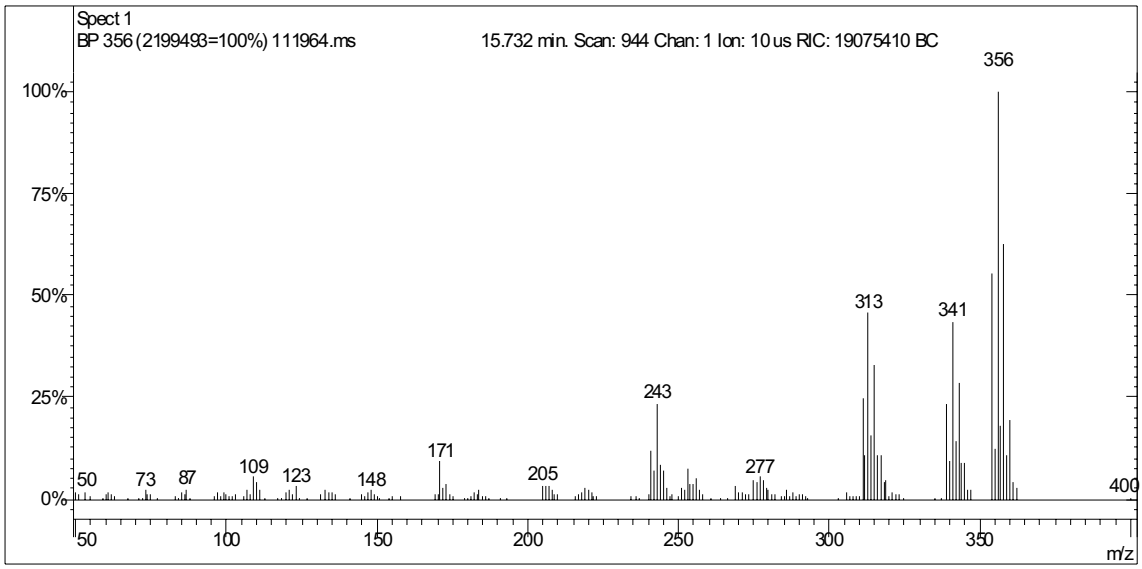

Figure A.10: 2',2,3',3,5-pentachloro-4-methoxybiphenyl GC/ Mass Spectrum

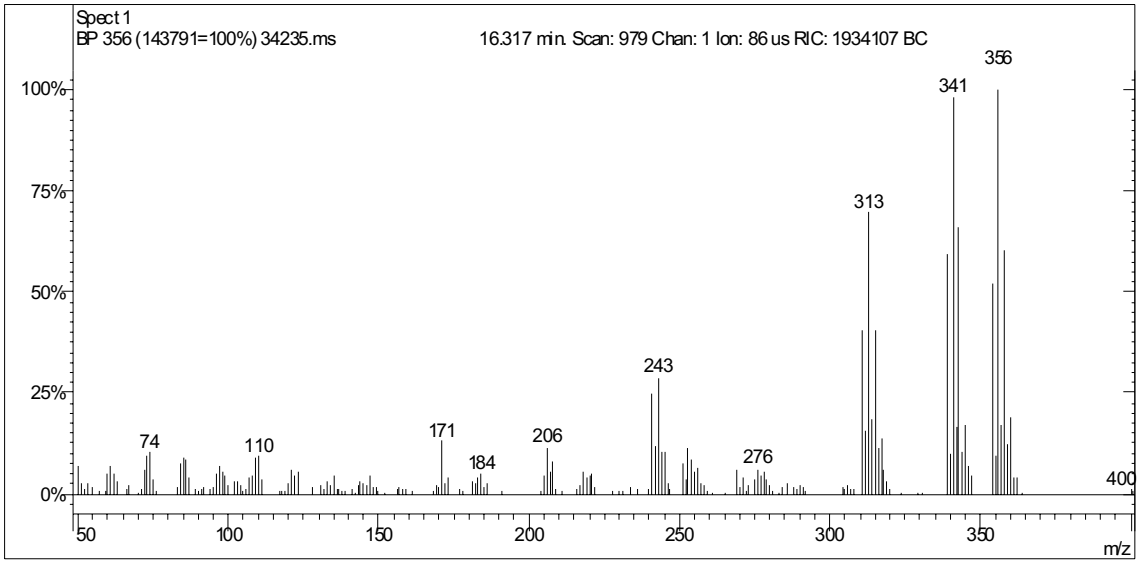

Figure A.11: 2,3',3,4',5-pentachloro-4-methoxybiphenyl GC/ Mass Spectrum

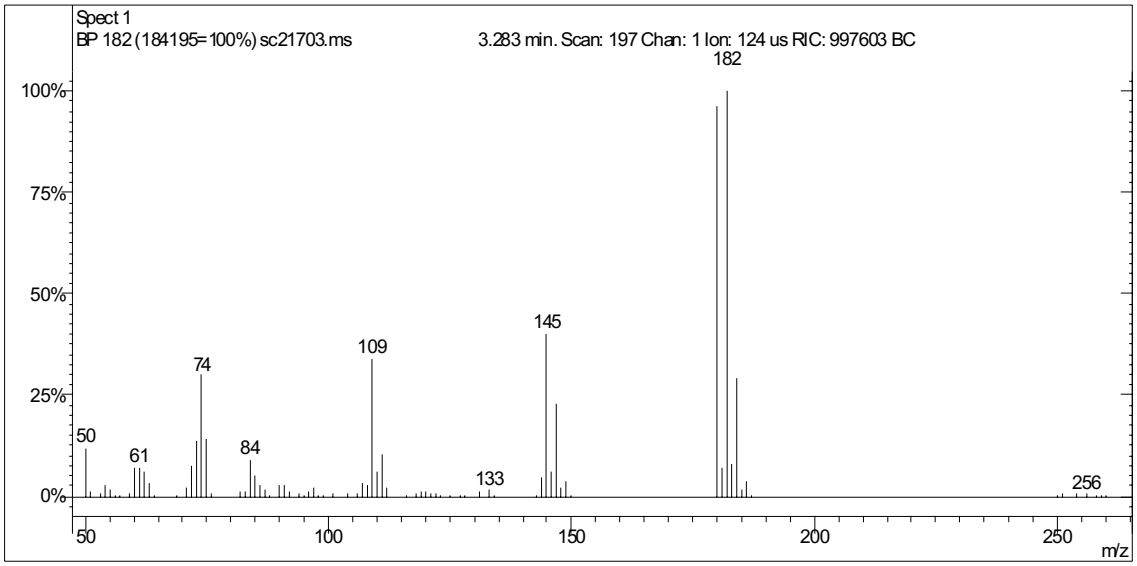

Figure A.12: Trichlorobenzene GC/ Mass Spectrum

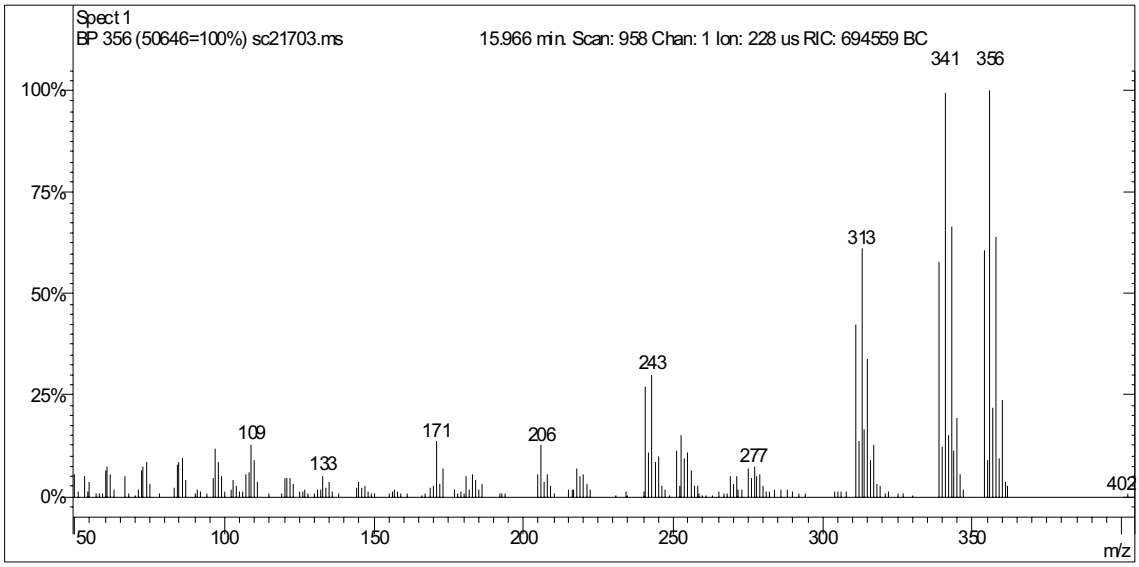

Figure A.13: 2',3',3,5',5- pentachloro-4-methoxybiphenyl GC/ Mass Spectrum

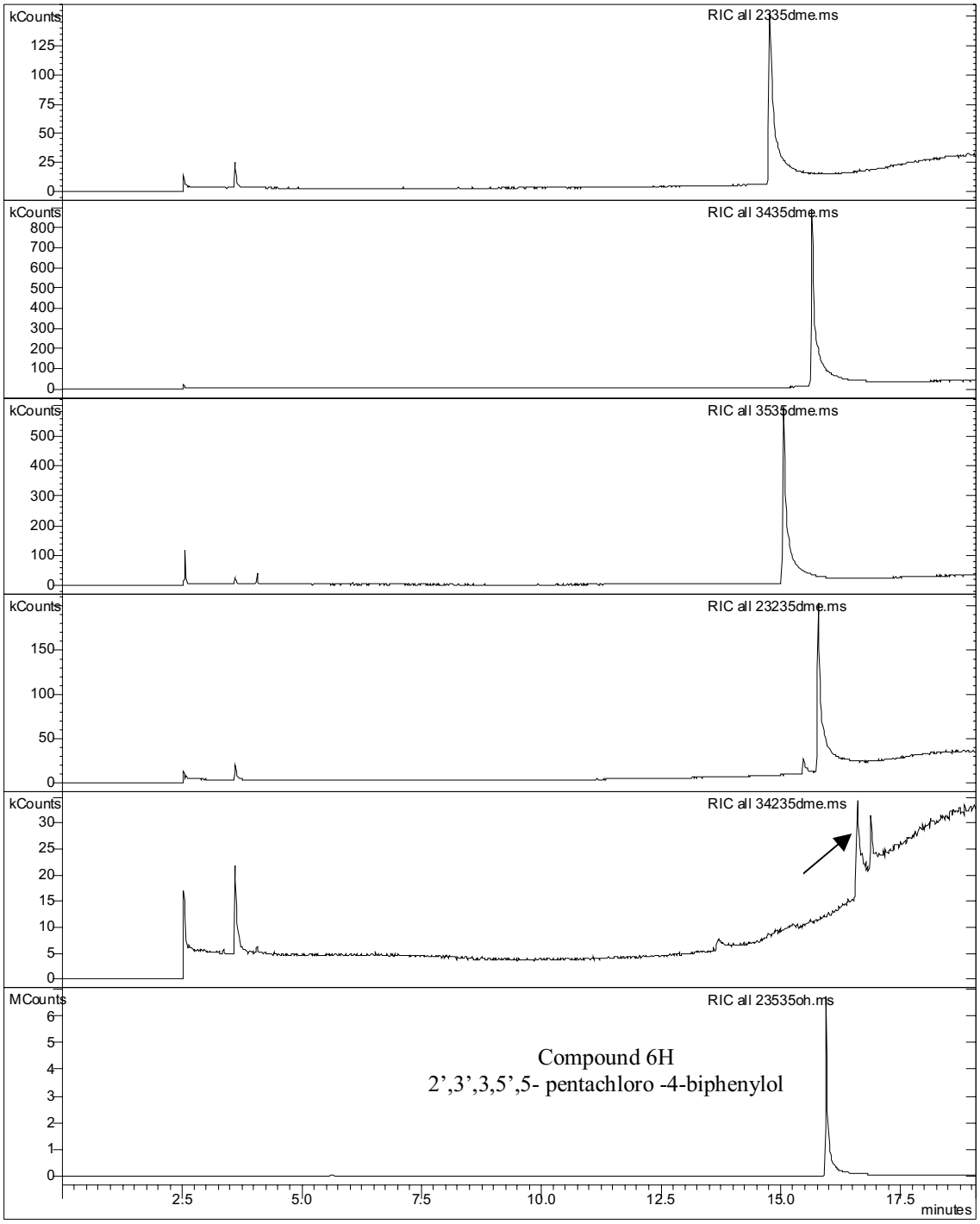

Figure A.14: GC Chromatograms of Polychlorobiphenylols 1H-6H

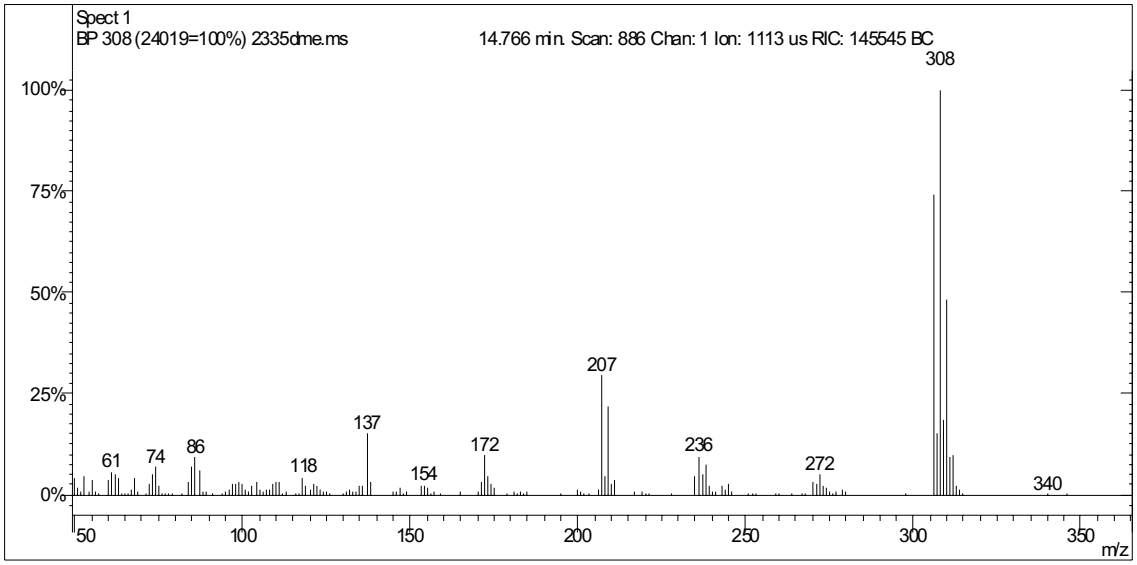

Figure A.15: 2',3',3,5-tetrachloro-4-biphenylol GC/ Mass Spectrum

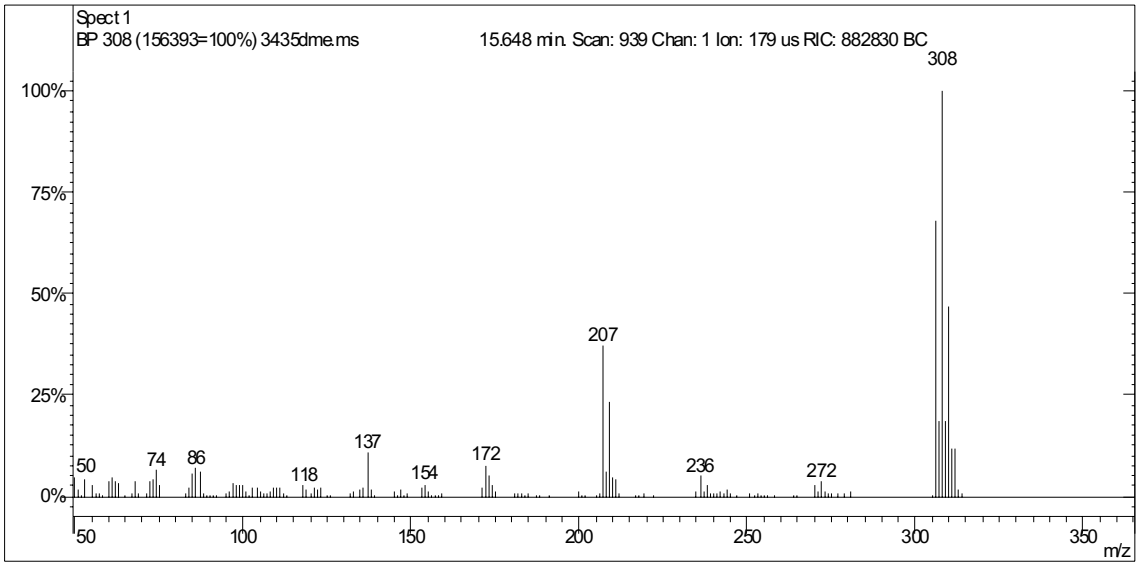

Figure A.16: 3',3,4',5-tetrachloro-4-biphenylol GC/ Mass Spectrum

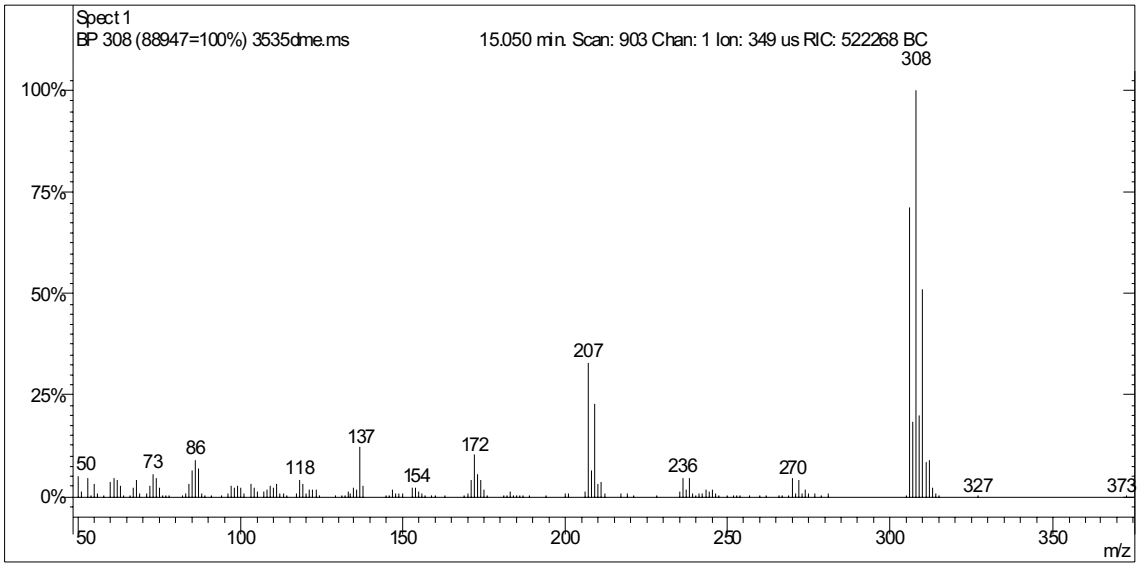

Figure A.17: 3',3,5',5-tetrachloro-4-biphenylol GC/ Mass Spectrum

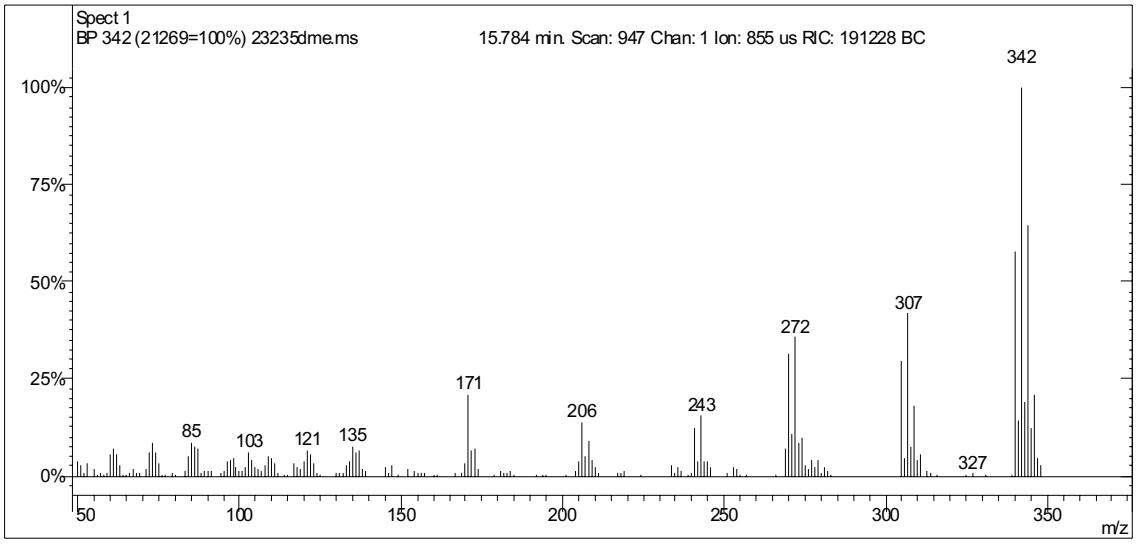

Figure A.18: 2',2,3',3,5- pentachloro -4-biphenylol GC/ Mass Spectrum

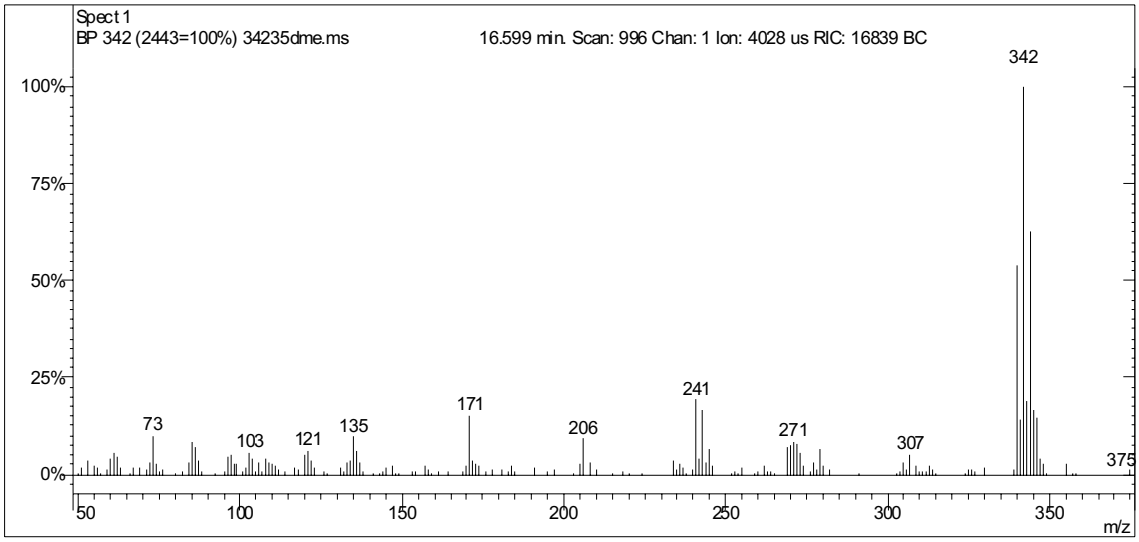

Figure A19: 2,3',3,4',5-pentachloro -4-biphenylol GC/ Mass Spectrum

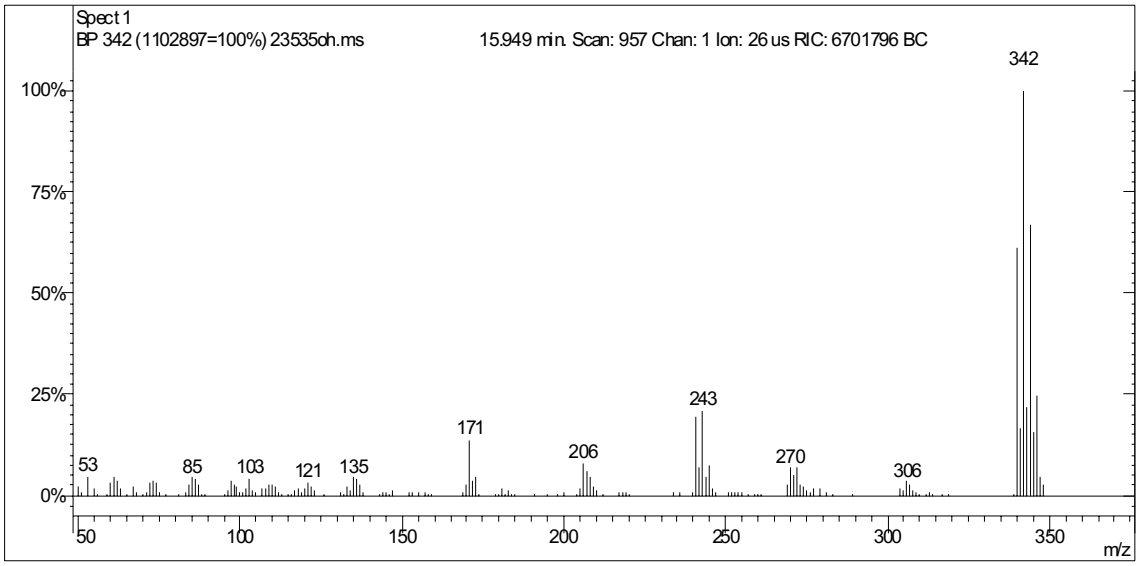

Figure A.20: 2',3',3,5',5- pentachloro -4-biphenylol GC/ Mass Spectrum

APPENDIX B

INFRARED SPECTROSCOPY DATA

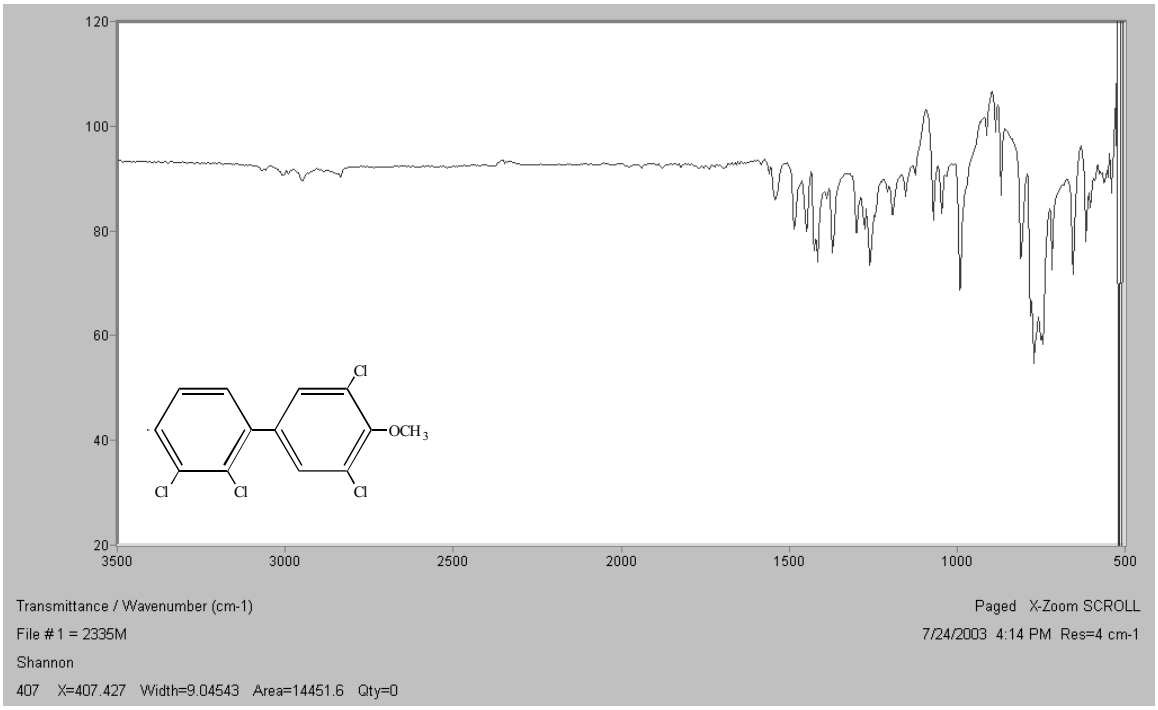

Figure B.1: Compound 1 IR Spectrum

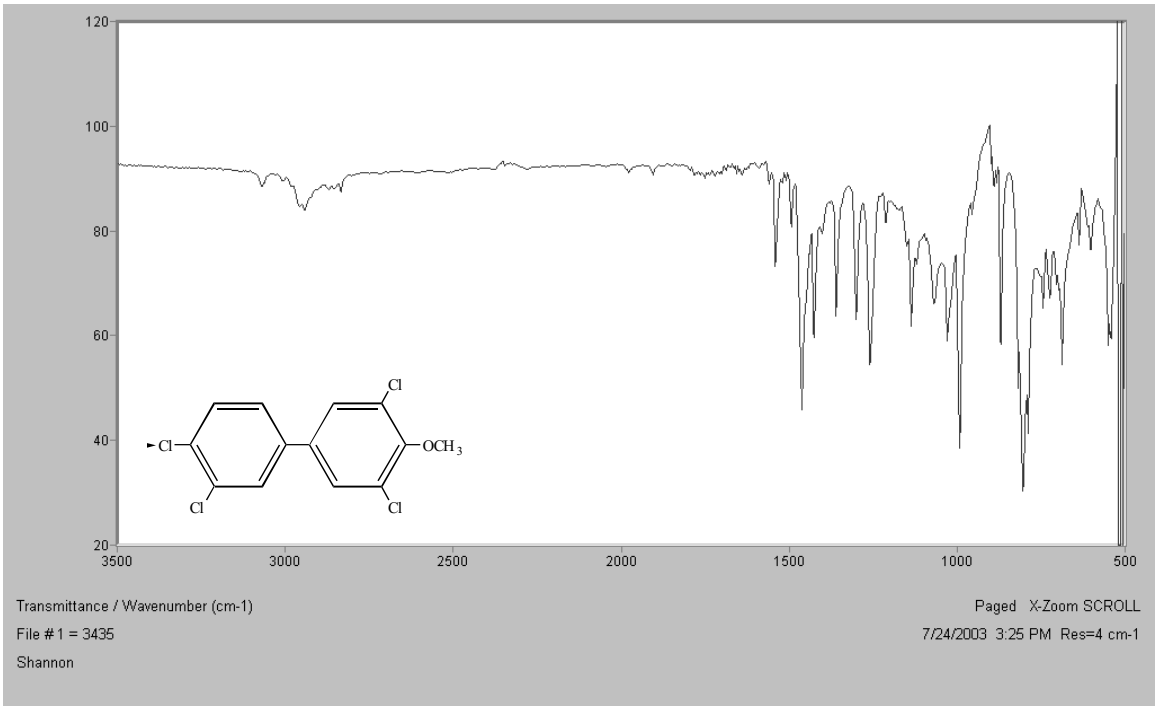

Figure B.2: Compound 2 IR Spectrum

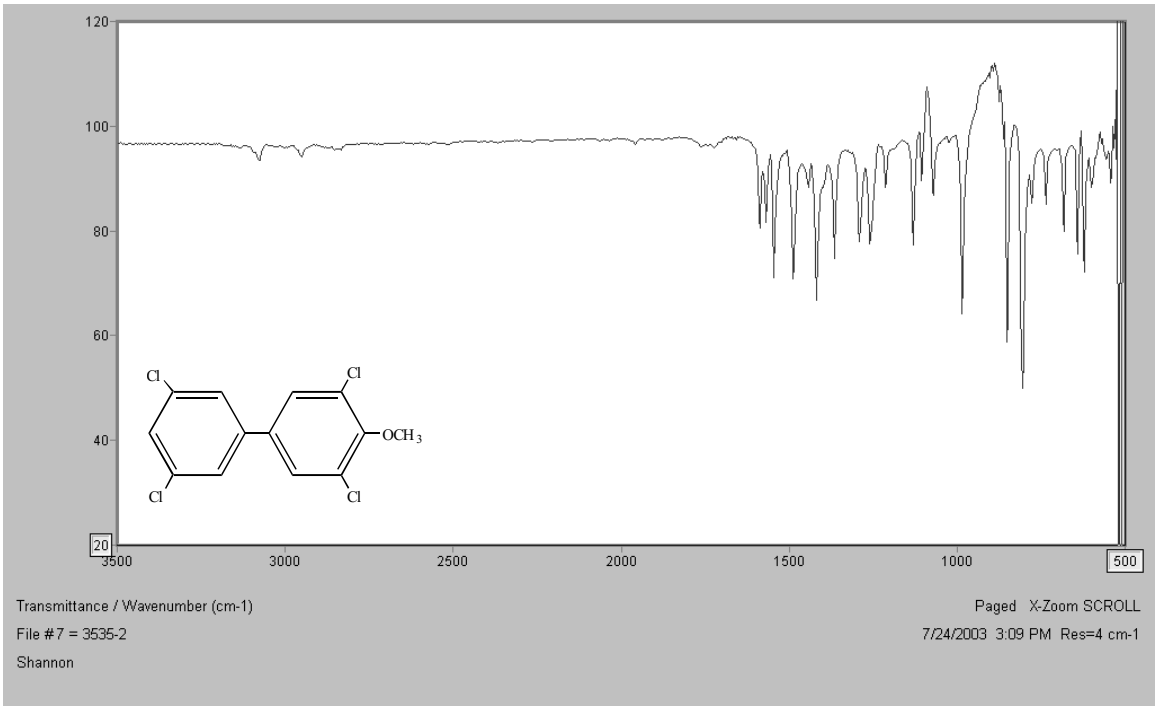

Figure B.3: Compound 3 IR Spectrum

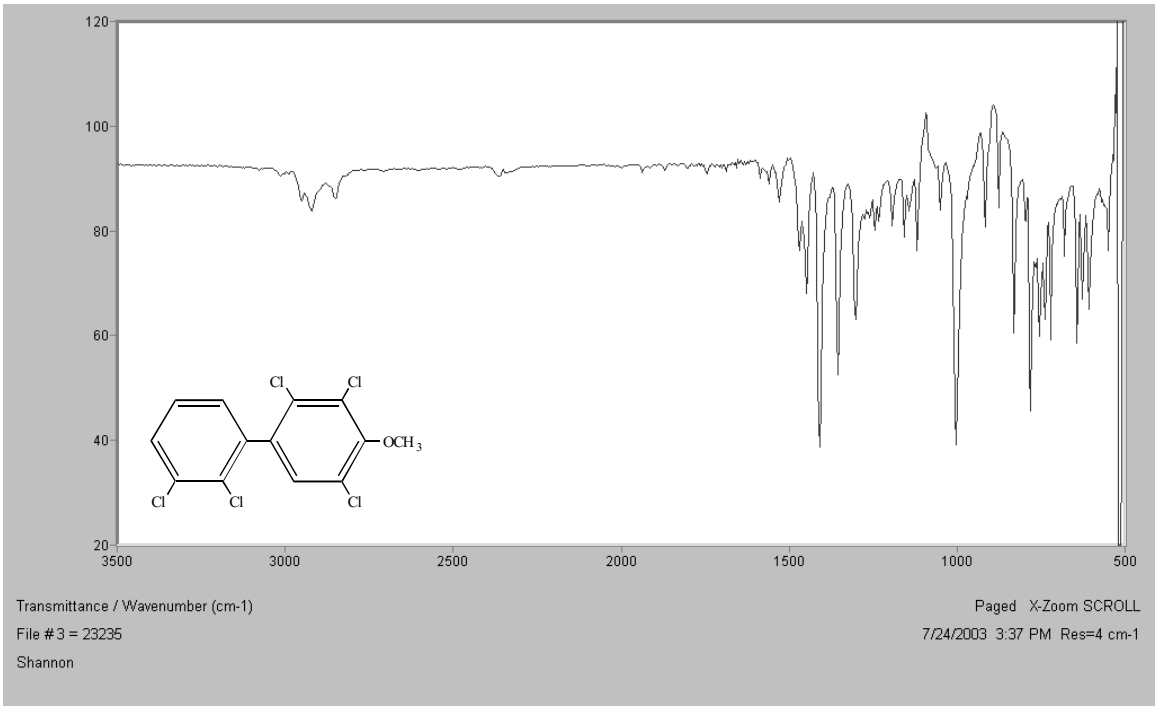

Figure B.4: Compound 4 IR Spectrum

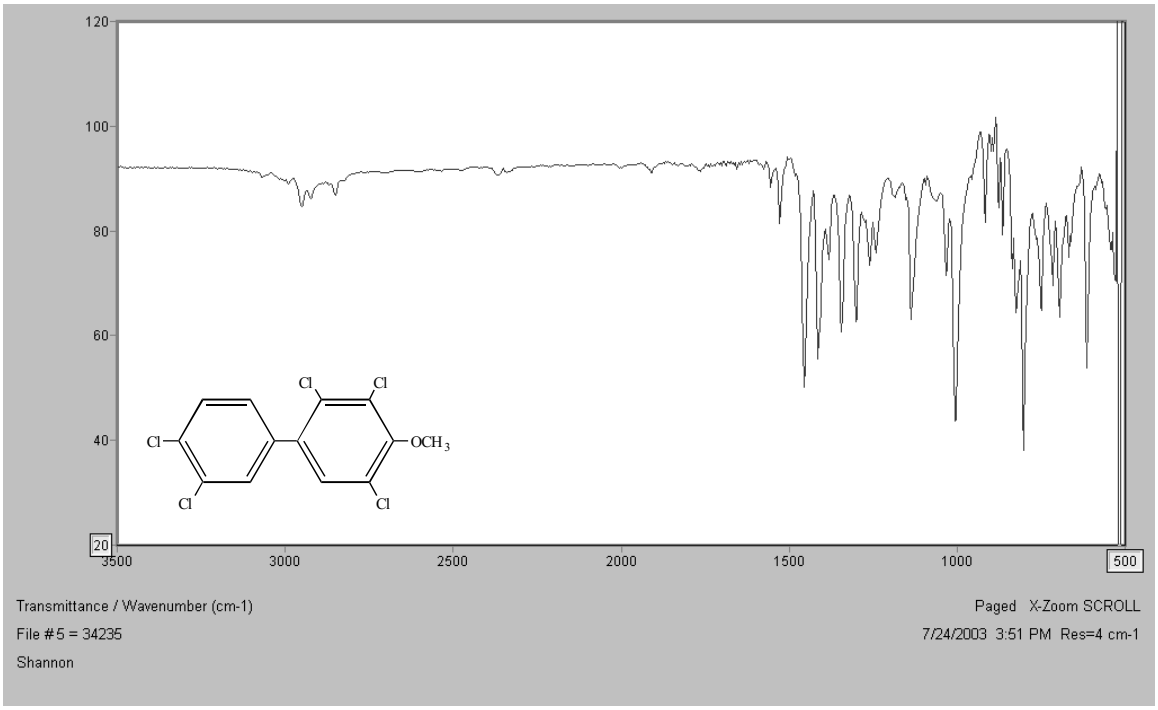

Figure B.5: Compound 5 IR Spectrum

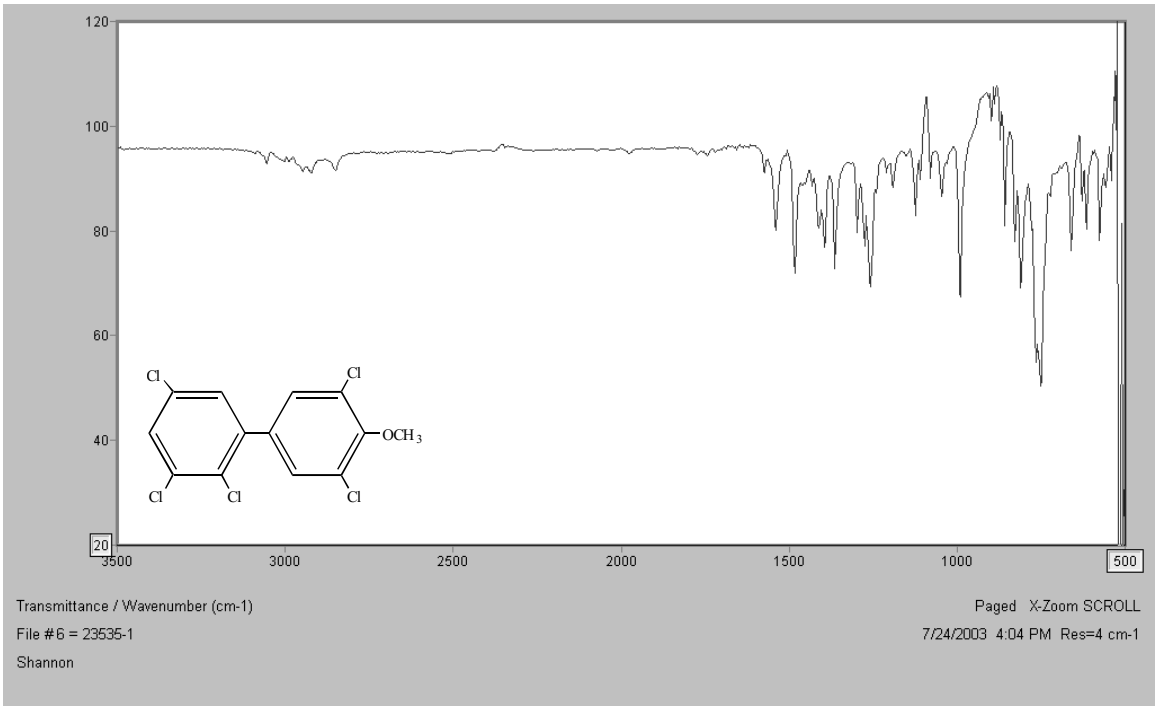

Figure B.6: Compound 6 IR Spectrum

Table B.1: Summary of IR Data for PCMBs

	PCMB Compound					
	1	**2**	**3**	**4**	**5**	**6**
Major Peaks (cm^{-1}) in the Ar-H region	3069	3070	3077	3014	3004	3055
	3059	3006	3002	2992	2991	3004
	3005	2959	2994	2952	2951	2990
	2992	2942	2952	2922	2923	2948
	2950	2870	2872	2851	2851	2922
	2836	2854	2853			2852
		2834	2835			
Major Peaks (cm^{-1}) in the fingerprint region	1416	1462	1544	1409	1455	1483
	1370	1258	1487	1001	1135	1257
	1260	992	1418	830	1004	990
	990	869	1355	781	801	750
	809	803	984	643	612	658
	772	685	850			
	653		805			
			621			

APPENDIX C

LIQUID CHROMATOGRAPHY/ MASS SPECTROMETRY DATA

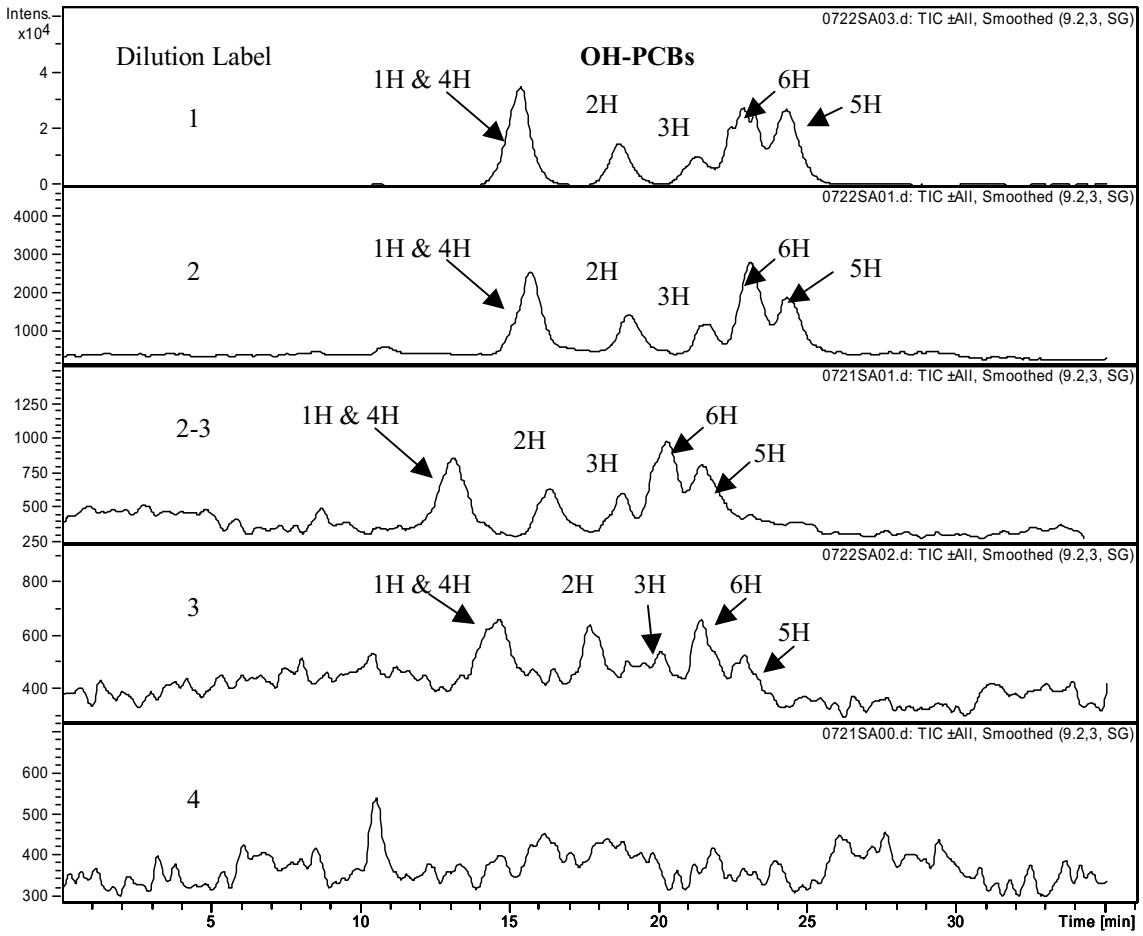

Figure C.1: LC Chromatograms of Decreasing Concentrations of OH-PCBs 1H-6H from Standard Curve Experiments in Acetonitrile

Figure C.1 contains LC chromatograms obtained from analyses of five acetonitrile solutions containing the six OH-PCB compounds. The five solutions were serial dilutions of a stock solution, see Table C.1 for the concentrations of each analyte. The data from these analyses were used to create standard curves for each of the six OH-PCBs.

Table C.1: Concentrations of OH-PCBs 1H-6H from Standard Curve Experiments in Acetonitrile

Dilution Label	Concentration in □g/mL					
	1H	2H	3H	4H	5H	6H
1	5	8	5.1	5.5	6.2	9.3
2	0.5	0.8	0.51	0.55	0.62	0.93
2-3	0.125	0.2	0.1275	0.1375	0.155	0.2325
3	0.05	0.08	0.051	0.055	0.062	0.093
4	0.005	0.008	0.0051	0.0055	0.0062	0.0093

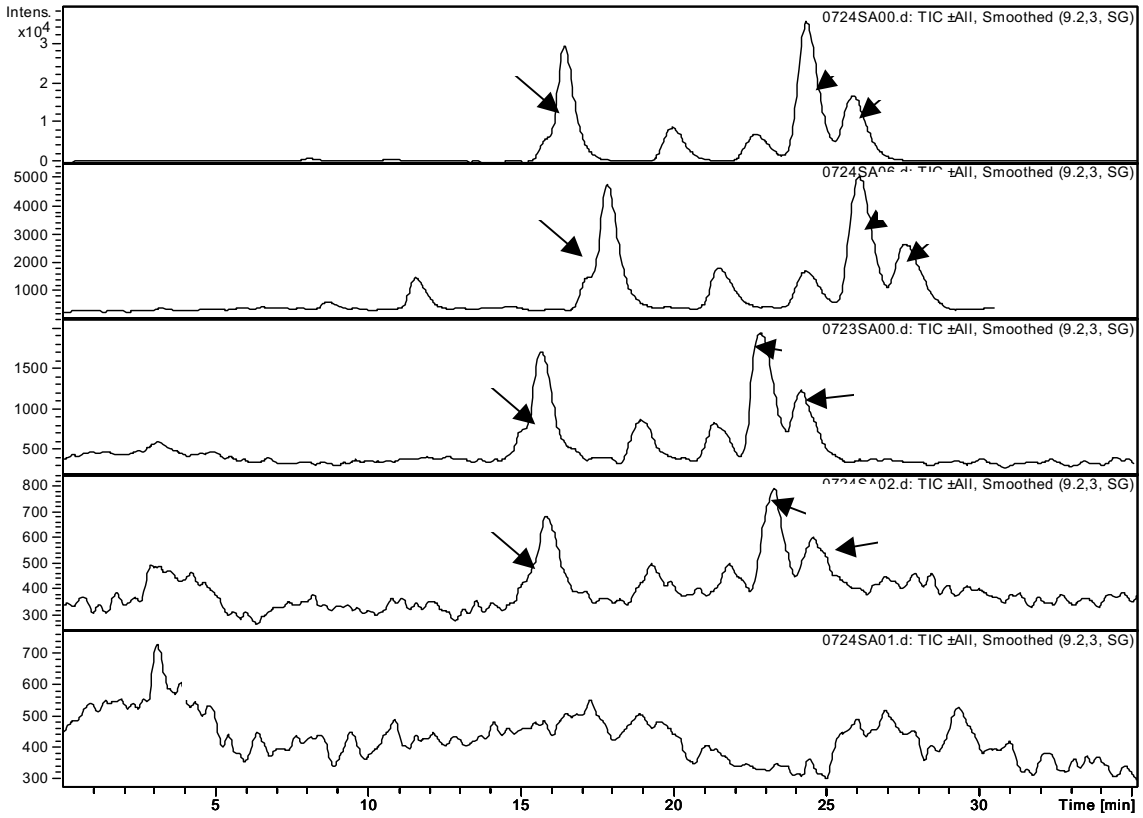

Figure C.2: LC Chromatograms of Decreasing Concentrations of OH-PCBs 1H-6H from Standard Curve Experiments in Water

Figure C.2 contains LC chromatograms obtained from analyses of five water solutions containing the six OH-PCB compounds. The five solutions were serial dilutions of a stock solution, see Table C.2 for the concentrations of each analyte. The data from these analyses were used to create standard curves for each of the six OH-PCBs.

Table C.2: Concentrations of OH-PCBs 1H-6H from Standard Curve Experiments in Water

Dilution Label	Concentration in □g/mL					
	1H	2H	3H	4H	5H	6H
1	0.5	0.8	0.51	0.55	0.62	0.93
1-2	□□□□	0.4	0.255	0.275	□□□□	0.465
2	0.05	0.08	0.051	0.055	0.062	0.093
2-3	0.0125	0.02	0.01275	0.01375	0.0155	0.02325
3	0.005	0.008	0.0051	0.0055	0.0062	0.0093

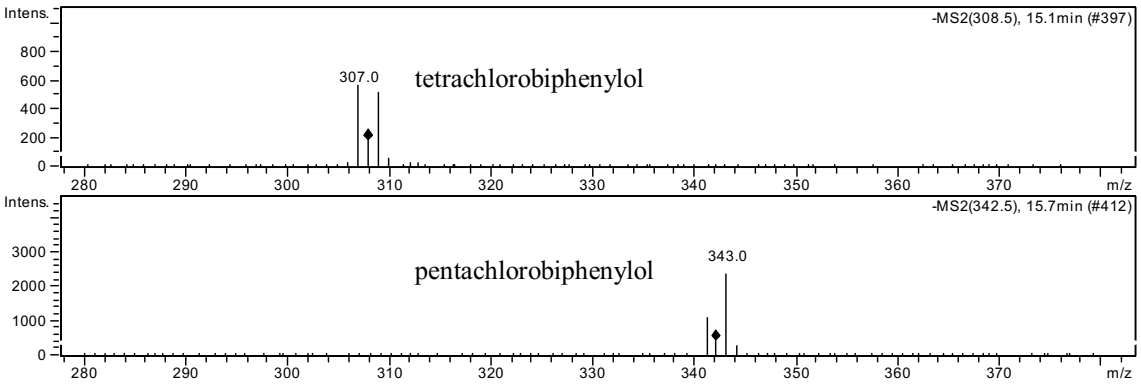

Figure C.3: LC/Mass Spectra of a Tetrachlorobiphenylol and a Pentachlorobiphenylol in Acetonitrile

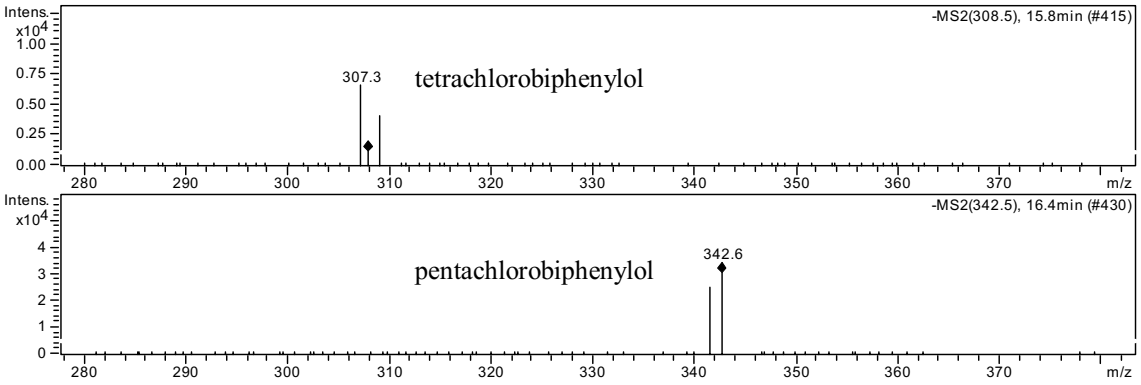

Figure C.4: LC/Mass Spectra of a Tetrachlorobiphenylol and a Pentachlorobiphenylol in Water

Figures C.3 and C.4 contain mass spectra where an ion was selected within a window of 3amu for detection of the OH-PCBs, 308.5 for the tetrachlorobiphenylol and 342.5 for the pentachlorobiphenylol. Analytes in Figure C.3 were in acetonitrile, and analytes in Figure C.4 were in water.

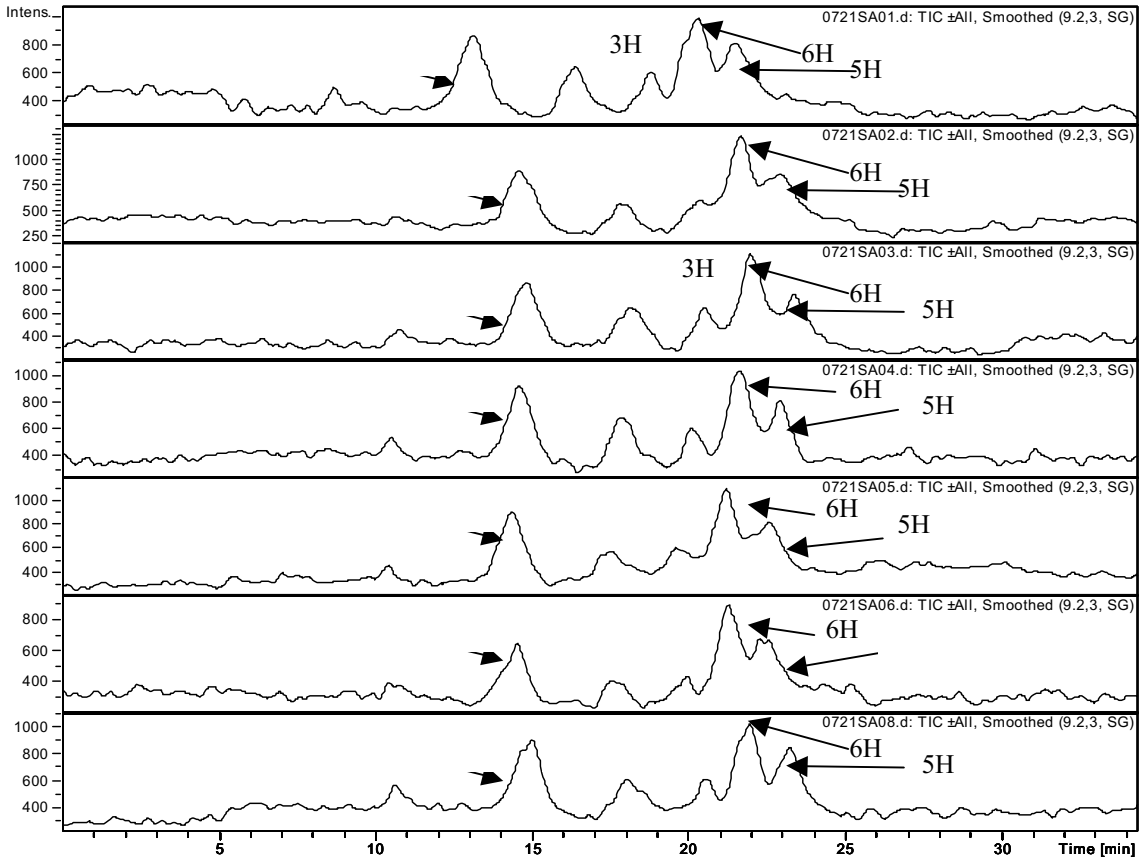

Figure C.5: LC Chromatograms of OH-PCBs 1H-6H from Reproducibility Studies in Acetonitrile

Figure C.5 contains seven LC chromatograms obtained from analyses of dilution 2-3 (in Table C.1) containing the six OH-PCB compounds. The data from these analyses were used to determine reproducibility for detecting each of the six OH-PCBs.

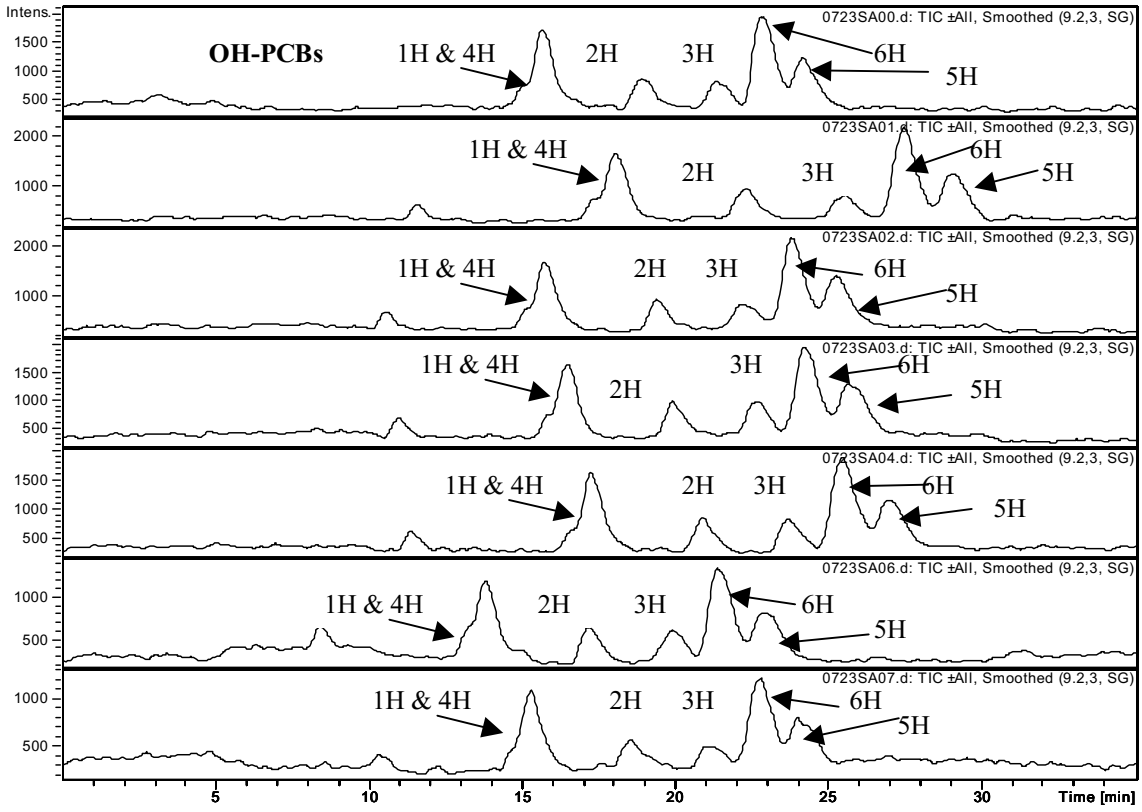

Figure C.6: LC Chromatograms of OH-PCBs 1H-6H from Reproducibility Studies in Water

Figure C.6 contains seven LC chromatograms obtained from analyses of dilution 2 (in Table C.2) containing the six OH-PCB compounds. The data from these analyses were used to determine reproducibility for detecting each of the six OH-PCBs.

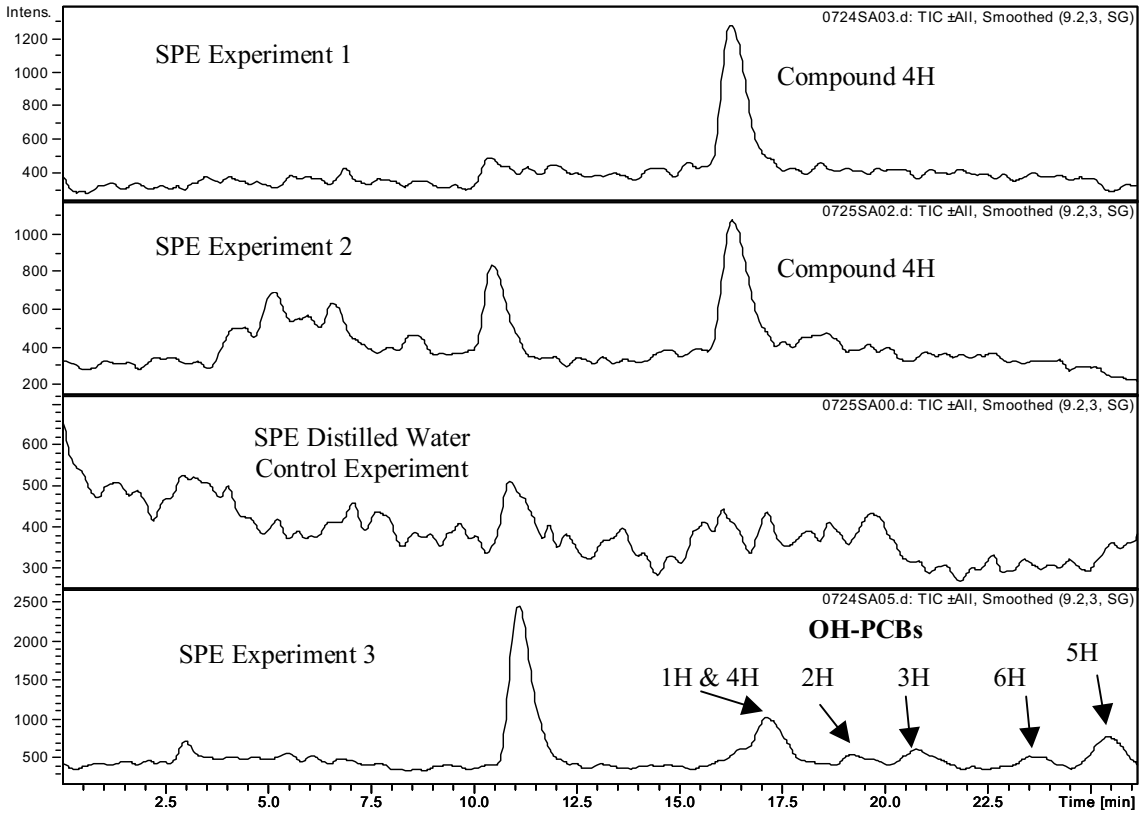

Figure C.7: LC Chromatograms of OH-PCBs from SPE Experiments 1, 2, 3, and Distilled Water Control

Figure C.7 contains LC chromatograms obtained from experiments coupling solid phase extraction with LC/ESI/MS. SPE experiments 1and 2 analyzed detection of compound 4H when it was spiked onto an SPE column. A control experiment was performed using only distilled water spiked onto an SPE column. SPE experiment 3 analyzed detection of the six OH-PCBs spiked onto an SPE column.

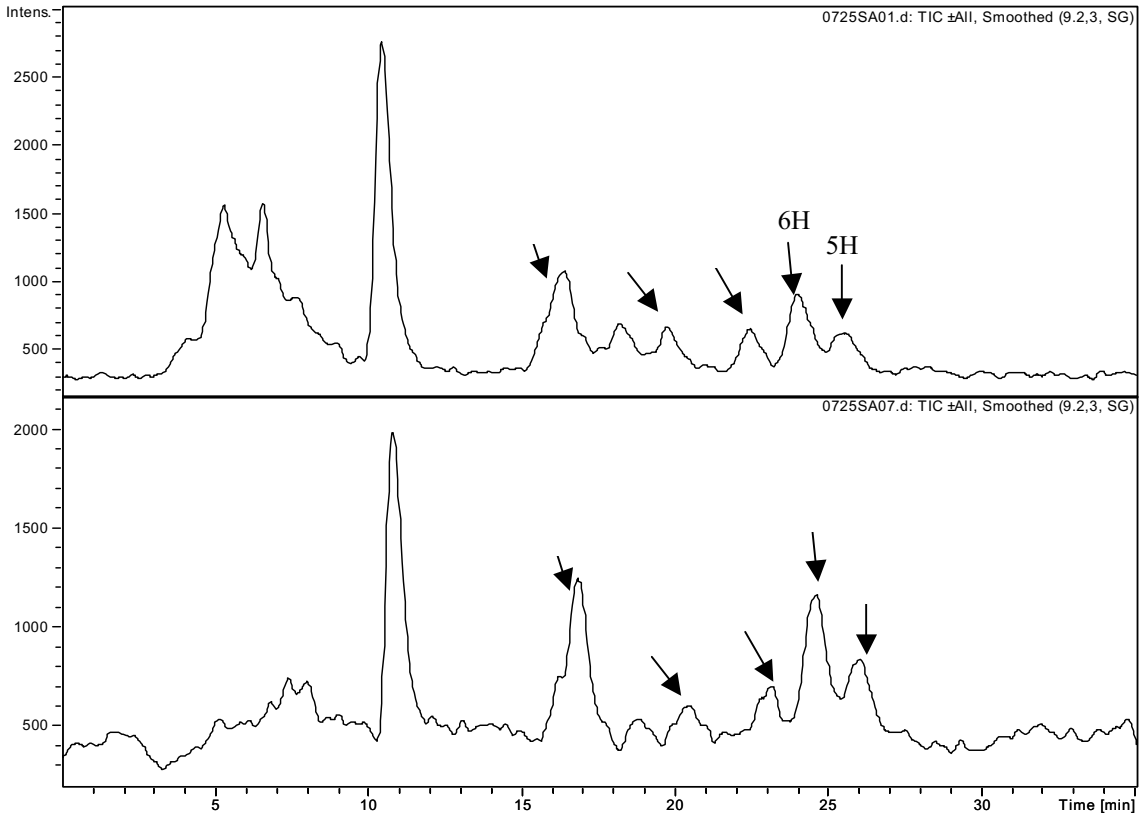

Figure C.8: LC Chromatograms of OH-PCBs 1H-6H from SPE Experiments 4-1 and 4-2

Figure C.8 contains LC chromatograms obtained from experiments coupling solid phase extraction with LC/ESI/MS. SPE experiments 4-1and 4-2 analyzed detection of all six OH-PCBs spiked into a distilled water solution and then extracted on an SPE column. Analytes in SPE experiment 4-1 (and all other SPE experiments except 4-2) were collected in a glass vial. Analytes in SPE experiment 4-2 were collected in a plastic vial.

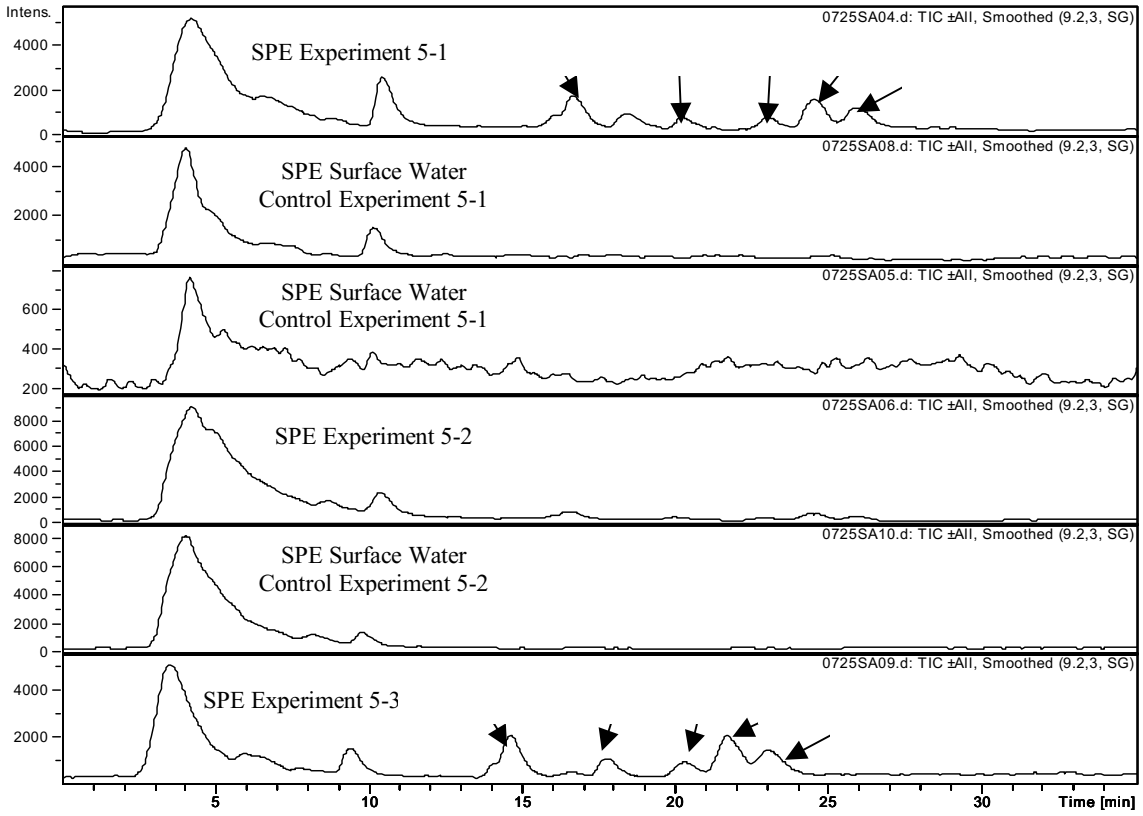

Figure C.9: LC Chromatograms of OH-PCBs 1H-6H from SPE Experiments 5-1, 5-2, 5-3, and Surface Water Controls

Figure C.9 contains LC chromatograms obtained from experiments coupling solid phase extraction with LC/ESI/MS. SPE experiments 5-1, 5-2, and 5-3 analyzed detection of all six OH-PCBs spiked into a surface water solution and then extracted on an SPE column. SPE experiments 5-1 and 5-2 were performed using surface water from different locations. SPE experiment 5-3 was performed using the same surface water as in experiment 5-1; however, the sample was not filtered (as it was for experiments 5-1 and 5-2) before extracting on the SPE column.